Skin Microbiome: The Impact of Hyperglycaemia, pH and Temperature on Antibiotic Susceptibility to Rifampicin, Clindamycin and Erythromycin in *S. aureus* and *S. epidermidis*

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Abbreviations

***S. aureus* / SA:** *Staphylococcus aureus*

***S. epidermidis* / SE:** *Staphylococcus epidermidis*

PS: Parent strain

P13: Adapted strain after 13 passages

SE(0): *Staphylococcus epidermidis* adapted strain at 0mm glucose

SE(5.5): *Staphylococcus epidermidis* adapted strain at 5.5mm glucose

SE(20): *Staphylococcus epidermidis* adapted strain at 20mm glucose

***E. coli*:** Escherichia coli

AMR: Antimicrobial resistance

MRSA: Methicillin-resistant *Staphylococcus aureus*

DF: Degrees of freedom

SD: Standard deviation

OTU: Operational taxonomic unit

DFU: Diabetic foot ulcer

AST: Antibiotic susceptibility testing

***P. acnes*:** *Propionibacterium acnes*

***S. lugdunesis*:** *Staphylococcus lugdunensis*

OoCs: Organs-on-chips

PoC: Point of care

DA: Clindamycin

RD: Rifampicin

E: Erythromycin

Abstract

In the recent decades, treating acute wounds, particularly in diabetic patients, has become a significant challenge in clinical settings due to the rising bacterial resistance. This study investigates the impact of hyperglycaemia, pH, and temperature on antibiotic susceptibility. Susceptibility testing of *S. aureus* (SGT20 04103) and *S. epidermidis* (SGT31) clinical isolates to Clindamycin (2µg), Rifampicin (5µg), and

Erythromycin (10µg) was conducted using the disk diffusion method according to the EUCAST guidelines. The testing was performed under various conditions: i) both strains were serially adapted for 13 passages at glucose concentrations of 0mm, 5.5mm, and 20mm; ii) the Rifampicin-adapted *S. epidermidis* strain (at all glucose concentrations) was further adapted to all three antibiotics at pH levels of 5.5, 7.5, and 8.5; iii) the same strain was adapted to all three antibiotics at temperatures of 33°C, 37°C, and 39°C as well. The results showed a negative correlation between antibiotic susceptibility and glucose, and positive correlations with pH and temperature. Additionally, there was a significant difference in susceptibility between the parent strain and the high-glucose-adapted strain, specifically under low pH and low temperature conditions (P_{DA}=0.0020, P_{RD}= 0.0002, P_E= 0.0136; and P_{DA}= 0.0019, P_{RD}<0.0001, P_E=0.0007, respectively). In conclusion, bacteria adapted to high glucose concentrations show significantly reduced antibiotic susceptibility under the low pH and temperature conditions typical of wound healing stage. Therefore, combination testing and treatment are required to understand bacterial resistance mechanisms and potentially personalize treatment based on the specific wound type and patient health conditions.

1. Introduction

1.1. Skin microbiome and wounds

Skin wounds are common issue in the clinical enviroment, and the increased risk of complications has become major challenge, especially for patients with diabetes (Burgess et al., 2021). Wound healing is a process that involves the interaction of different types of cells and growth factors, in order to restore the affected tissue (Ellis et al., 2018). However, there are external factors that play a crucial role in this restoration, such as the skin microbiota (Zheng et al., 2022). Recent research has shown that commensal bacteria drive innate wound healing response of the skin (Di Domizio et al., 2020; Wanke et al., 2011). Therefore, the interactions between the host immune system and skin microbiome, promote faster skin repair.

1.2. Staphylococcus epidermidis

Staphylococcus epidermidis is one of the most abundant bacteria found in the human skin microbiome (Otto, 2009). Additionally, it is known that all *S. epidermidis* isolates

behave similarly (Otto, 2009). However, recent evidence suggests that different strains can either benefit or harm the skin barrier (Cau et al., 2021; Conlan et al., 2012; Zhou et al., 2020). Furthermore, *S. epidermidis* serves as a potential reservoir of antibiotic resistance genes (Tang et al., 2020; Xue et al., 2017). Recent data show that *S. epidermidis*-derived Lipopeptide 78 (LP78) reduces skin inflammation to aid wound healing, indicating that LP78 could be a promising treatment for delayed or non-healing wounds (Li et al., 2019). Therefore, treatment efficacy is influenced not only by the medications, but also by the bacteria. Further investigation into these bacteria is necessary, as they may form the basis for new wound healing therapies (Serra et al., 2015).

1.3. Staphylococcus aureus

From the other hand, *Staphylococcus aureus* is a widespread bacterial pathogen causing numerous skin infections and hundreds of thousands to millions of severe infections globally each year (Rasigade et al., 2014). It is one of the four most prevalent bacterial species found in chronic wounds (Roy et al., 2020). Its biofilm formation results in challenging infection management and treatment (Halbert et al., 1992; Roy et al., 2020). Further, the multidrug methicilin resistance of *S. aureus* (MRSA) poses a major threat to patient treatment and has led to extensive research on its genetic basis, evolution, and spread (Jensen & Lyon, 2009). Extensive research over the past few decades has focused on preventing biofilm formation (Akbas & Kokumer, 2015; López et al., 2019). This includes the use of botanical drugs, derivatives, and inhibitors to enhance antibiotic susceptibility (Czarnecka et al., 2020; Handzlik et al., 2013; Miladiyah & Rachmawaty, 2017; Quave et al., 2012). Additionally, alternative treatments to antibiotics are necessary, as this species has undergone significant mutations, rendering common treatments less effective (Samir et al., 2022). Therefore, a deeper understanding of resistance mechanisms is essential to develop novel and more effective treatments.

1.4. Diabetes

1.4.1 Pathophysiology of diabetes

Type 1 and Type 2 diabetes are the most common forms of diabetes and are primarly caused by both genetic and environmental factors (Imayama et al., 2011). Recent research has identified genetic *loci* that cause dysfunction of the β-cells, therefore

affecting the insulin secretion or action (Franks et al., 2008; Holmkvist et al., 2006; Willer et al., 2007). Additionally, a rise in diabetes cases has been observed in the recent years, due to rapid environmental changes, causing epigenetic drifts (Candler et al., 2018). Type 1 diabetes is caused by an autoimmune destruction of the β-cells, B cells, and macrophages (commonly due to MHC (Major Histocompatibility Complex) alterations) and unknown environmental factors (Tesauro & Mazzotta, 2020). However, type 2 diabetes is caused by a combination of genetic predipositions and lack of physical activity, obesity, and aging (Meneilly, 2000). Diabetes is characterized by increased blood glucose levels, or hyperglycaemia, and results in protein, fat, and carbohydrate metabolic dysfunctions (Giacco & Brownlee, 2011).

1.4.2. Complications of Type 2 diabetes

Diabetes mellitus Type 2 accounts for 95% of the cases worldwide and remains clinically inevident (Packer et al., 2024). Due to the abnormal glucose metabolism, therefore chronic hyperglycaemia, there are many macro- and microvascular complications (Packer et al., 2024). Macrovascular complications include cardiovascular and cerebrovascular diseases, while microvascular complications involve neuropathies, nephropathy and retinopathy (Fox et al., 2004; Wei et al., 2009). However, diabetic ulcers, remain a severe and common complication, which affects the overall health and quality of life of the patients (Muduli et al., 2015). Research has shown that 5% of patients with infected new ulcers have to undergo major amputation in one year (Prompers et al., 2008). Furthermore, data show five-year mortality rates of 45% and 55%, for patients with neuropathic and ischemic ulcers respectively (Moulik et al., 2003). Such rates are proven to be similar or worse than those of breast, prostate and colon cancers (Jemal et al., 2017; Jupiter et al., 2016; Wukich et al., 2017). Diabetes foot infections are usually treated with antibiotics- oral, topical or parenteral (Edmonds & Foster, 2004). However, the severity of the wound and the susceptibility of the bacterial burden affect the efficasy of the treatment (Dang et al., 2003; Hartemann-Heurtier et al., 2004; Lipsky et al., 2012). Studies suggest that clinicians must focus on the antibiotic resistance and reassess the antibiotic selection in order to prevent the emergence of resistant strains (AW et al., 2015; Kathirvel et al., 2018; Xie et al., 2017).

1.5. Antibiotics and resistance

1.5.1. Adaptation

Bacteria are able to develop resistance through adaptation (Bedhomme et al., 2019). This means that consistent interactions with antibiotics allow bacteria to develop defence mechanisms against the antibiotics, through mutation and natural selection (Oz et al., 2014). Therefore, resistance occurs due to genetic mutations, which allows the bacteria to grow and multiply even in the presence of antibiotic that would normally kill them (Spagnolo et al., 2016). This leads to treatment failure and the need of alternative treatment options (Gjini & Brito, 2016). Such example is the methicillin-resistant *Staphylococcus aureus* (MRSA) (Muhlebach et al., 2017). From the other hand there is the phenomenon of bacterial persistence, which is a temporary state where a small bacterial population is able to survive antibiotic treatment, without being genetically resistant to the specific antibiotic (Germain et al., 2013). Persistence is often due to phenotypic changes, where the bacterial persisters enter a slow-growing state, which makes them less susceptible to antibiotics, which target actively growing cells (Wakamoto et al., 2013). Therefore, the persisters are able to survive antibiotic treatment, and when the treatment is stopped, they will be able to repopulate and cause a potential infection relapse (Hu et al., 2015). Such example is the persister cells in biofilms, as in chronic *Staphylococcus epidermidis* bacterial infections (Yang et al., 2015). Recent research has examined these two phenomena, bacterial resistance and persistence, and it suggests that they are complimentary, but independent (Vogwill et al., 2016). Specifically, different strains of *Pseudomonas* were tested against the antibiotics ciprofloxacin and rifampicin, and the results showed positive correlation between resistance and persistence across the different strains (Vogwill et al., 2016). However, they found different genes that controlled these two phenomena, leading to the conclusion that they are independent traits (Vogwill et al., 2016). Therefore, these differing genes and control mechanisms might indicate different phenotypes, which are susceptible to different types of cellular stress-different treatment approach (Vogwill et al., 2016).

1.5.2. Beta-lactam antibiotics

The most common treatments, used for wound healing, are clinically prescribed antibiotics (Duong et al., 2010; Khan et al., 2014). Between 53.3% and 71% of patients

receive a wound-related antibiotic at some stage during their outpatient wound treatment (Howell-Jones et al., 2006; Öien & Forssell, 2013; Price, 2020). However, antibiotic resistance has become increasingly prevalent in the last years. Antibiotic resistance in both *Staphylococcus aureus* and *Staphylococcus epidermidis* is majorly attributed to their biofilm formed on the surface of infected tissues (Chen et al., 2019; Roy et al., 2020). Additionally, both species are methicillin resistant, therefore reduced affinity of beta-lactam antibiotics (Hiramatsu et al., 2013; Lee et al., 2018). Beta-lactam antibiotics, which include penicillins, cephalosporins, monobactams, and carbapenems, are primarily used to treat bacterial infections (Pandey & Cascella, 2019). They work by interfering with the synthesis of the bacterial cell wall, leading to the death of the bacteria (Pandey & Cascella, 2019). The most harmful species are the β-lactamase producing bacteria, because this enzyme provides resistance to beta-lactam antibiotics (Hussain et al., 2021). These genes exist in various sequences within these bacteria, explaining why *Staphylococcus* species are recognized as some of the most predominant beta-lactam resistant organisms (Mirhoseini et al., 2016). However, although bacteria can develop resistance naturally, the improper use of the antibiotics has affected this phenomenon as well (Andreatos et al., 2018). Resistant bacteria are being selected and have the ability to grow even in the presence of the specific antibiotic (Jernigan et al., 2020).

1.5.3. Mutations

Mechanisms of horizontal gene transfer between bacterial strains or species are frequently considered as the primary drivers of antibiotic resistance (Händel et al., 2014). The emergence of antibiotic resistance through *de novo* acquisition is associated with particular mutations and the altered expression of specific genes (Andersson & Hughes, 2010; Martínez & Rojo, 2011; Toprak et al., 2012). Furthermore, bacterial resistance can be readily induced by gradual exposure of the bacteria to sublethal concentrations of antibiotics (van der Horst et al., 2011). Prolonged exposure to antibiotics activates the SOS response (DNA repair mechanisms), leading to resistance-causing mutations (Beaber et al., 2004; Händel et al., 2013). Nevertheless, history and chance significantly influence the development of new resistance phenotypes, the creation of collateral sensitivity networks, the degree of evolved resistance, and the predictability of the eventual resistance phenotype (Gifford et al., 2018; Pál et al., 2015; Scribner et al., 2020; Vogwill et al., 2014). Recent study showed

that evolution of antibiotic resistance is dependent on bacterial lifestyle and environmental structure (Santos-Lopez et al., 2019). Santos-Lopez and colleagues tested biofilm and planktonik *Acitenobacter* populations against ciprofloxacin, and observed that biofilm populations developed genotypes and phenotypes with lower resistance, along with collateral sensitivity to β-lactam drugs (Santos-Lopez et al., 2019).

1.5.4. Antibiotic's mode of action: Rifampicin, Clindamycin, Erythromycin

Staphylococcus aureus and *Staphylococcus epidermidis* are common Gram-positive bacteria that form biofilms (Pérez-Prieto et al., 2019). Therefore, antibiofilm antibiotics are considered very effective against these species (Pérez-Prieto et al., 2019). Rifampicin is known to penetrate their biofilms effectively, however it is important to be combined with another antibiotic to prevent resistance (Achermann et al., 2013; Zimmerli et al., 1998). Furthermore, a study has shown that a combination of manuka honey and rifampicin has the potential to stop the appearance of rifampicin-resistant *S. aureus in vitro*, therefore suggesting that it may be a novel therapy for chronic wounds particularly in diabetic patients (Müller et al., 2013). Clindamycin is an antibiotic that binds to the bacterial ribosomes and inhibit protein synthesis (Kasten, 1999). Therefore, clindamycin is primarily bacteriostatic, meaning that inhibits the growth and reproduction of the species, rather than directly killing them (Pankey & Sabath, 2004). With the relatively high prevalence of patients with penicillin allergy, clindamycin offers safe and effective alternative to first-line antibiotics (beta-lactams) (Baxter et al., 2020; Huether et al., 2002). Recent study demonstrated that a combination of topical insulin with clindamycin effectively reduce inflammation and accelerate full-thickness wound healing (Mirhoseini et al., 2021). Additionally, Scheinfeld showed that a combination treatment of rifampicin and clindamycin offers the ideal treatment for hidradenitis suppurativa (HS), a chronic inflammatory skin condition, characterized by inflammation and blockage of hair folicles and sweat glands (Scheinfeld, 2016). Erythromycin is a broad-spectrum antibiotic with similar mode of aftion to clindamycin (Bunch & Mcguire, 1953; Liang & Han, 2013). It inhibits the bacterial protein synthesis, however when binding to the ribosomes, blocks the translocation of the peptides, instead of interfering with their bond formation (Heilman et al., 1952; Liang & Han, 2013). Bioactive wound dressing loaded with erythromycin has been discovered in 2016, and has been proved to behave as drug reservoir,

providing continuous antibiotic release to the infected wound (de Souza et al., 2016). Therefore, this membrane could effectively protect the wound site and inhibit bacterial proliferation (de Souza et al., 2016). Furthermore, erythromycin is proven effective against persistent facial acne lesions alone, or in a combination with intense pulled light (IPL) or zinc (Al-Hamamy et al., 2014; Faghihi et al., 2012). Recent research indicates that combining erythromycin with honey shows promising inhibitory effects against *S. aureus*, and enhances overall wound healing (Fathollahipour et al., 2020).

1.5.5. Antibiotic resistance

Antibiotics are proven for many years to help and improve the process of skin regeneration (Diehr et al., 2007). However, recent studies have shown that antibiotics often have negative effects on the host microbiome (Zhang et al., 2015). Their inability to penetrate the bacterial biofilms, makes them uncapable to efficiently kill and stop the bacterial growth (Brown & Poston, 1983; Kurokawa et al., 1988; Walsh & Wencewicz, 2014). Therefore, leading to multi-drug antimicrobial resistance (AMR), due to their increased defence against antibiotics. Furthermore, recent research shows that the expansion of AMR is caused by the substantial changes that the use of systemic antibiotics induces (Jo et al., 2021). Key changes are reduction in microbial diversity, overgrowth of opportunistic pathogens, changes in microbial community composition, impact on skin immune function, and long-term or even permanent alterations (Rogers et al., 2014; Zhu et al., 2021). Furthermore, variations in the strain- and species- levels of the microbiome are proven to be a marker for clinical outcomes and therapeutic efficasy, specifically in diabetes patients (Kalan et al., 2019). Recent study shows that glucose increases biofilm formation, and therefore antibiotic resistance (She et al., 2019). Their research is the first to suggest correlation between biofilm formation and glucose, identifying novel targets to counteract biofilm formation, and therefore resistance (She et al., 2019).

1.6. pH and wound treatment

Changes of the skin pH impact the effectiveness of antimicrobials, influencing their performance in wound healing environments (Lengheden & Jansson, 1995; P. Sim et al., 2022). Current scientific evidence shows that pH is important for both healing and treatment of chronic and acute wounds (Pivian Sim et al., 2022). Furthermore, it is proven that during the healing process, pH progresses from an alkaline state to neutral

and then acidic state (Leveen et al., 1973; Pivian Sim et al., 2022). The reason for this is that alkaline environment is more conducive to bacterial burden (Percival et al., 2014; Weinrick et al., 2004). Therefore, alkalinity of a wound will increase to further optimise bacterial growth (Percival et al., 2014; Weinrick et al., 2004). Research shows that the pH value affects the effectiveness of antibiotics (Mercier et al., 2002). Specifically, antibiotic's activity decreases significantly in an acidic environment, however toxicity strongly increases in an alkaline milieu, resulting in 90-fold higher efficasy compared to acidic pH values (Mercier et al., 2002). In the case of the raising MRSA, where the multi-resistant bacteria are very difficult to tackle, a new therapy might include wound treatment with a shift of the pH milieu (Dissemond et al., 2002; Dissemond et al., 2005).

1.7. Temperature and wound treatment

Temperature affects wound healing and treatment effectiveness in diabetic patients (Brooks et al., 2021). A research from 2015 demonstrates a positive correlation between wound bed temperatures and improved wound conditions in patients with venous leg ulcers (Dini et al., 2015). After the wound formation, the bacterial colonisation causes local vasodilation, therefore increased temperature, which results in the delivery of more oxygen and nutrients to the wound (Gojiro Nakagami et al., 2010; Sun et al., 2001). Temperatures between 33°C–35°C indicates healing wound process, however there is insufficient evidence to support the use of temperature monitoring to adequately indicates wound healing progress (Dini et al., 2015; G Nakagami et al., 2010). Recent research shows that management of wound pH, temperature and bacterial burden, results in enhanced wound healing (Derwin et al., 2023). Therefore, a combined treatment approach is needed to overcome antibiotic resistance. Very recent study proposes a pH- and temperature-responsive hydrogel as a treatment for bacterial infections and improved wound healing (Haidari et al., 2022). This *in vivo* research demonstrated that this antibacterial hydrogel effectively cure *Staphylococcus aureus* wound infections and significantly accelerate their wound healing rate (Haidari et al., 2022).

1.8. Aim and objectives of current research

Wound management in diabetic patients has become huge challenge in the last decades. However, there is no research that has examined the specific relationship between bacterial antibiotic susceptibilty and glucose concentrations. Furthermore, there is no specific study that suggests a correlation between antibiotic susceptibility and different pH and temperature conditions. Therefore, if glucose, pH and temperature actually affect antibiotic ssusceptibility, this finding will give novel insights of wound treatment, especially in diabetic patents. The aim of this research is to examine the relationship between antibiotic susceptibility and glucose levels. The objectives are to test skin bacteria (*S. aureus* and *S. epidermidis*) and perform serial adaptation to three antibiotics (Rifampicin, Clindamycin, and Erythromycin) through passaging under different conditions (glucose, pH level, and temperature). Specifically, the conditions will include: i) glucose concentrations of 0%, 5.5%, and 20%; ii) pH levels of 5.5, 7.5, and 8.5; iii) temperatures of 33°C, 37°C, and 39°C. The hypothesis of this research is that higher glucose levels result in faster adaptation and lower antibiotic susceptibility (Hsu et al., 2015; Xiang et al., 2023). To further examine this concept, the initial parent strains of the bacteria will be compared to the very last adapted strains under different pH and temperature conditions. The expected result is a negative correlation between glucose, temperature, pH and antibiotic susceptibility (Li et al., 2020; McArdle et al., 2018).

2. Materials and Methods

2.1. Bacteria

Staphylococcus aureus (SGT20 04103) and *Staphylococcus epidermidis* (S5T36A TMS; SGT31) are clinical isolates kindly donated by Delphine Gilrich and Dr Thierry Naas at Hôpital Bicêtre (Hôpitaux Universitaires Paris-Sud) to Dr Joe Latimer at The University of Salford, as part of a collaborative research project.

2.2. Antibiotics

The antibiotics used for this experiment were Amoxicillin (30 µg), Clarithromycin (15 µg), Ampicillin (10 µg), Vancomycin (10 µg), Doxycycline (10 µg), Cefoxitin (30 µg), Erythromycin (15 µg), Rifampicin (5 µg), Clindamycin (2 µg, 10 µg), Tobramycin (10 µg), Levelfloxacin (5 µg), Meropenem (10 µg), Trimethoprim (5 µg), Ertopenem (10

µg), Doripenem (10 µg), Imipenem (10 µg), Cefpodoxime (10 µg), Ceftazidime (10 µg), Ceftriaxone (30 µg). The discs were obtained from Mast Diagnostics and the antibiotic concentrations were determined by availability at the time (Mast Group Ltd., 2024).

2.3. Agar and glucose

Mueller-Hinton (MH) agar was prepared according to the manufacturer's instructions (Merck, 2024). D--glucose powder (45.04 gr) was used to prepare 250 mL of 1 mol glucose solution (Thermo Scientific™ Remel, 2024). To achieve the desired glucose concentrations of 5.5% and 20%, 5.5 mL and 20 mL glucose was dilluted in 1 L of agar respectively. Three different media (0% (control), 5.5%, and 20%) were poured into Petri dishes (90 mm) with a level of depth 4.0 ± 0.5 mm. Plates were stored in clean fridge at 8-10°C.

2.4. Preparation of inoculum

Streak plates were prepared from the original clinical isolates (*S. aureus* (SGT20 04103) and *S. epidermidis* (S5T36A TMS; SGT31). Parent strains were incubated at 37°C for 24h. Phosphate buffered saline (PBS) was used and prepared according to the manufacturer's instructions (Thermo Scientific™ Remel, 2024). Direct colony suspension method was used to make a suspension of both bacteria in PBS to the density of 0.5 McFarland turbidity standard (equivalent to 1-2 x 108 CFU/mL for *Escherichia coli*) (Thermo Scientific™ Remel, 2024). Using a sterile metal loop, the desired colonies were picked up and suspended in 5mL PBS. The density of the suspention was visually adjusted to the 0.5 McFarland turbidity standard, using visual comparison card (Thermo Scientific™ Remel, 2024).

2.5. Inoculation of agar plates

Sterile cotton swab was used to inoculate the agar plates with the prepared suspensions following the EUCAST protocol (EUCAST, 2024). Separate suspensions were made for each plate and glucose concentration. As using Gram-positive bacteria, the swab was dipped into the suspension, without having to remove excess fluid before inoculation. Three directional swabbing method was used, to ensure evenly covered agar surface.

2.6. Application of antibiotic disks

Antibiotic disks were applied to the inoculated plates following the EUCAST protocol (EUCAST, 2024). Disks were applied firmly to tha surface of the agar within 15 minutes of the plate's inoculation. In case of misplacing the antibiotic, the disk was left at its initial place, as the initial diffusion of antimicrobial agents from disks is very rapid. Plates were incubated for 24h at 37°C.

2.7. Serial passage of bacteria

Serial passage experiment was conducted using the clinical isolates *S. aureus* (SGT20 04103) and *S. epidermidis* (S5T36A TMS; SGT31). First step was to find three antibiotics, which both strains were susceptable to. The initial chosen strains *S. aureus* (SGT20 04103) and *S. epidermidis* (S5T36A TMS) were inoculated on agar plates of 0%, 5.5% and 20% glucose concentrations, and tested against five antibiotics (Amoxicillin, Clarithromycin, Ampicillin, Vancomycin, Doxycycline). To measure the zone of inhibition and decide antibiotic susceptability/resistance, the EUCAST susceptability testing protocol and clinical breakpoints were followed (EUCAST, 2024). *S. epidermidis* (S5T36A TMS) showed complete resistance to all five antibiotics. Further nine antibiotics (Cefoxitin, Erythromycin, Rifampicin, Clindamycin, Tobramycin, Levelfloxacin, Meropenem, Trimethoprim, Ertopenem) were tested against both strains. *S. epidermidis* (S5T36A TMS) showed resistance to the second set of antibiotics as well. Therefore, the experiment continued with the initial *S. aureus* (SGT20 04103) strain, which showed susceptability to most of the antibiotics, and a new *S. epidermidis* strain (SGT31). The bacteria were tested against three chosen antibiotics of the second set (Erythromycin, Rifampicin, Clindamycin). Both showed good susceptibility, therefore the passages continued with these three antibiotics. After the first disk diffusion, sample from the edge of the inhibition zones of all plates were taken and suspended separately. Suspensions were inoculated on three different glucose concentration plates again, and antibiotic disks were placed one on each plate. Plates were incubated for 24h-48h at 37°C. For each passage, 18 plates were needed (2 strains, 3 glucose concentrations and 3 antibiotics). The same process was repeated for 12 passages. Between passages, inhibibition zones were measured and recorded. Passages were stopped earlier if complete resistance occurred faster. First passage was repeated, along with an additional 13th passage, making triplicates. The

first resistant strain *S. epidermidis* (S5T36A TMS) was tested against additional five antibiotics (Doripenem, Imipenem, Cefpodoxime, Ceftazidime, Ceftriaxone) to further assess its resistance profile.

2.8. pH testing

Mueller-Hinton (MH) agar (pH 7.3 ± 0.2) was prepared according to the manufacturer's instructions (Merck, 2024). Before autoclaving, the pH of the agar was altered to 5.5 and 8.5, using 1M HCL and 1M NaOH respectively. The pH level was measured using pH meter. After autoclaving, pH strips were used to recheck the pH level, using small amount of the agar poured on a petri dish, to avoid contamination. Samples of the parent strain *S. epidermidis* (SGT31), and its 13th passage with Rifampicin, were inoculated in triplicates on agar plates with pH levels 5.5, 7.5, and 8.5. These strains were tested against three antibiotics each time (Rifampicin, Erythromycin, Clindamycin). Plates were incubated for 24h at 37°C.

2.9. Temperature testing

Mueller-Hinton (MH) agar was prepared according to the manufacturer's instructions (Merck, 2024). Samples of the parent strain *S. epidermidis* (SGT31), and its 13th passage with Rifampicin, were inoculated in triplicates on agar plates three times. These strains were tested against three antibiotics each time (Rifampicin, Erythromycin, Clindamycin). Plates were incubated for 24h at 33°C, 37°C, and 39°C.

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism 10. Paired T-test was performed to test the significant correlation between the inhibition zones (adaptation) and glucose levels for the first and last passage. This was performed separately for each strain. The same test was performed for the results from the pH and temperature testing for *S. epidermidis.* All assays were performed in triplicates. Results were presented using column bar charts, with error bars and P-values displayed where applicable. Standard deviations and degrees of freedom were calculated using the same software.

Line chart graphs were plotted to show the trend of the adaptation throughout the passages for each antibiotic and three glucose concentrations. Excel (Office 365) was

used for this purpose. This was repeated for both species. Same line chart trends were plotted for the pH and temperature testing. Breakpoints were included.

3. Results

3.1. Relationship between adaptation and glucose concentrations

3.1.1. Adaptation of *S. aureus* and *S. epidermidis* on clindamycin, rifampicin and erythromycin altered the antibiotic susceptibility at three glucose levels

S. aureus and *S. epidermidis* were serially passaged 13 times on clindamycin, rifampicin and erythromycin, on three different glucose concentrations, to select for mutants with reduced susceptibility. Complete resistance to rifampicin at all three glucose concentrations was observed for *S. aureus* following the 4^{th} passage (Figure.1, Figure.2), and for *S. epidermidis* following the 6^{th} passage (Figure.3, Figure.4). No change in the susceptibility phenotypes was observed through the 13 passages. There were <2-fold changes in clindamycin and erythromycin susceptibility for both species following the 13 passages (Figure.5; Figure.6; Figure.7; Figure.8).

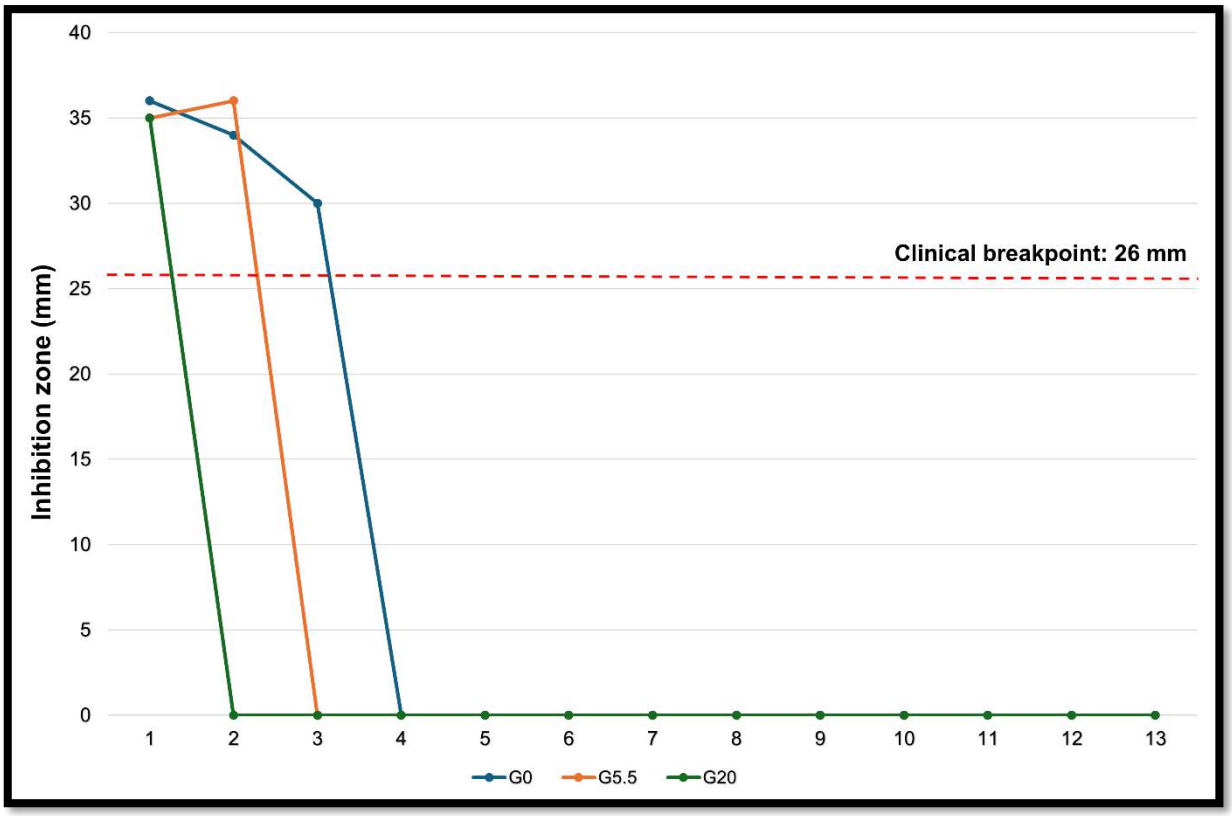

Figure. 1 *S. aureus* adaptation to Rifampicin (5µg) within the 13 passages. X axe showing the inhibition zone sizes and Y axe the number of passages. Blue line shows the adaptation on 0mm glucose (G0); Orange line shows the adaptation on 5.5mm glucose (G5.5); Green line shows the adaptation on 20mm glucose (G20). Red dashed line shows the clinical breakpoint for *S. aureus* on Rifampicin. The graph shows that complete resistance occurred in the 2nd passage at G20, in the 3rd passage for G5.5 and in the 4th passage for G0.

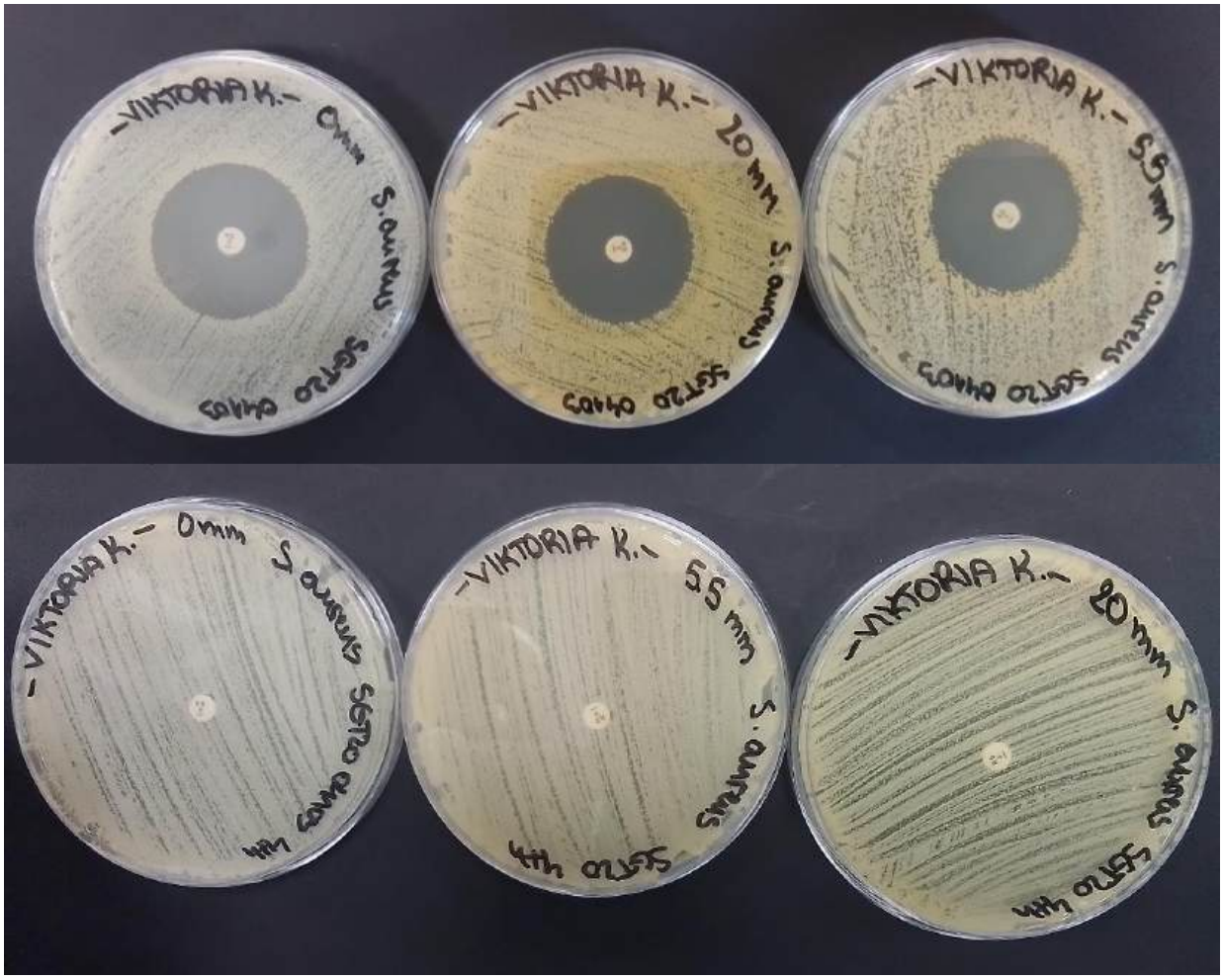

Figure. 2 Pictures showing the complete adaptation (resistance) of *S. aureus* strain to Rifampicin after 4 passages at all glucose concentrations.

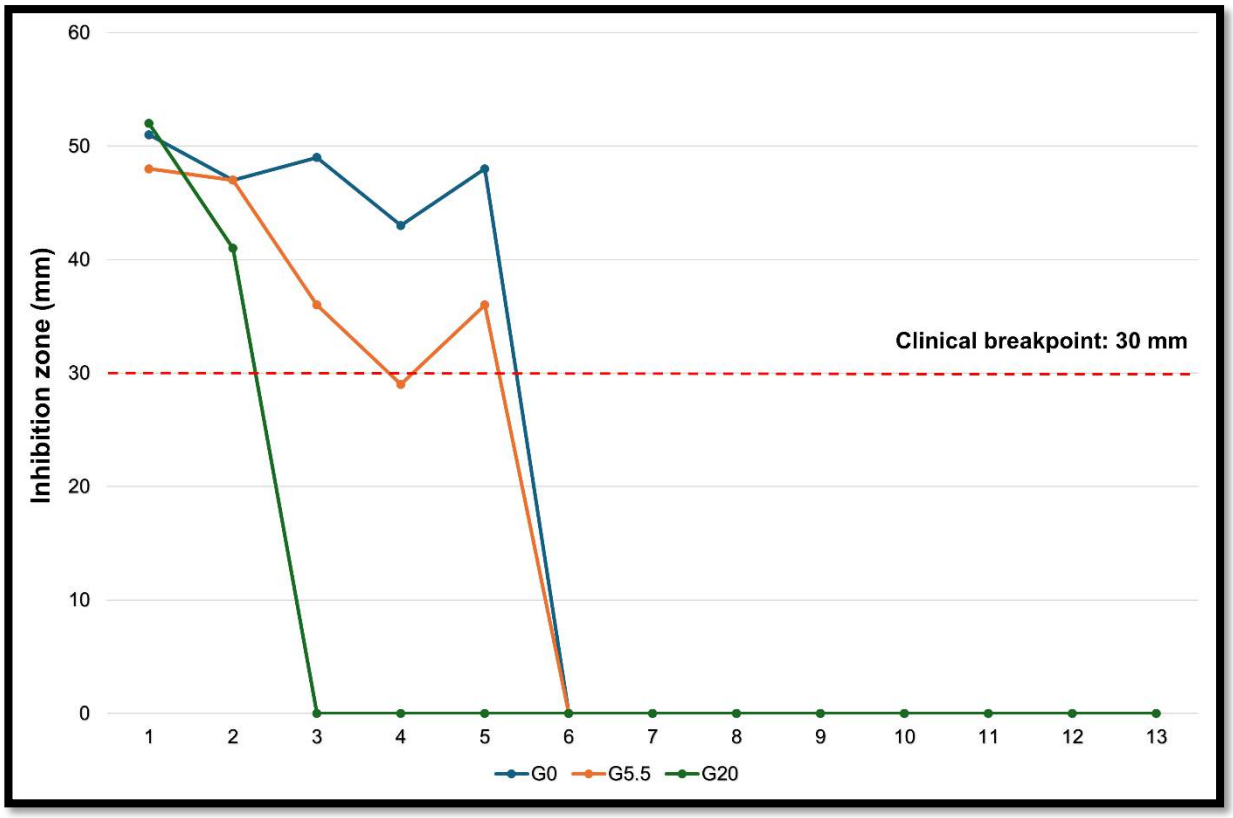

Figure. 3 *S. epidermidis* adaptation to Rifampicin (5µg) within the 13 passages. X axe showing the inhibition zone sizes and Y axe the number of passages. Blue line shows the adaptation on 0mm glucose (G0); Orange line shows the adaptation on 5.5mm glucose (G5.5); Green line shows the adaptation on 20mm glucose (G20). Red dashed line shows the clinical breakpoint for *S. epidermidis* on Rifampicin. The graph shows that complete resistance occurred in the 3rd passage at G20, in the 6th passage for G5.5 and for G0.

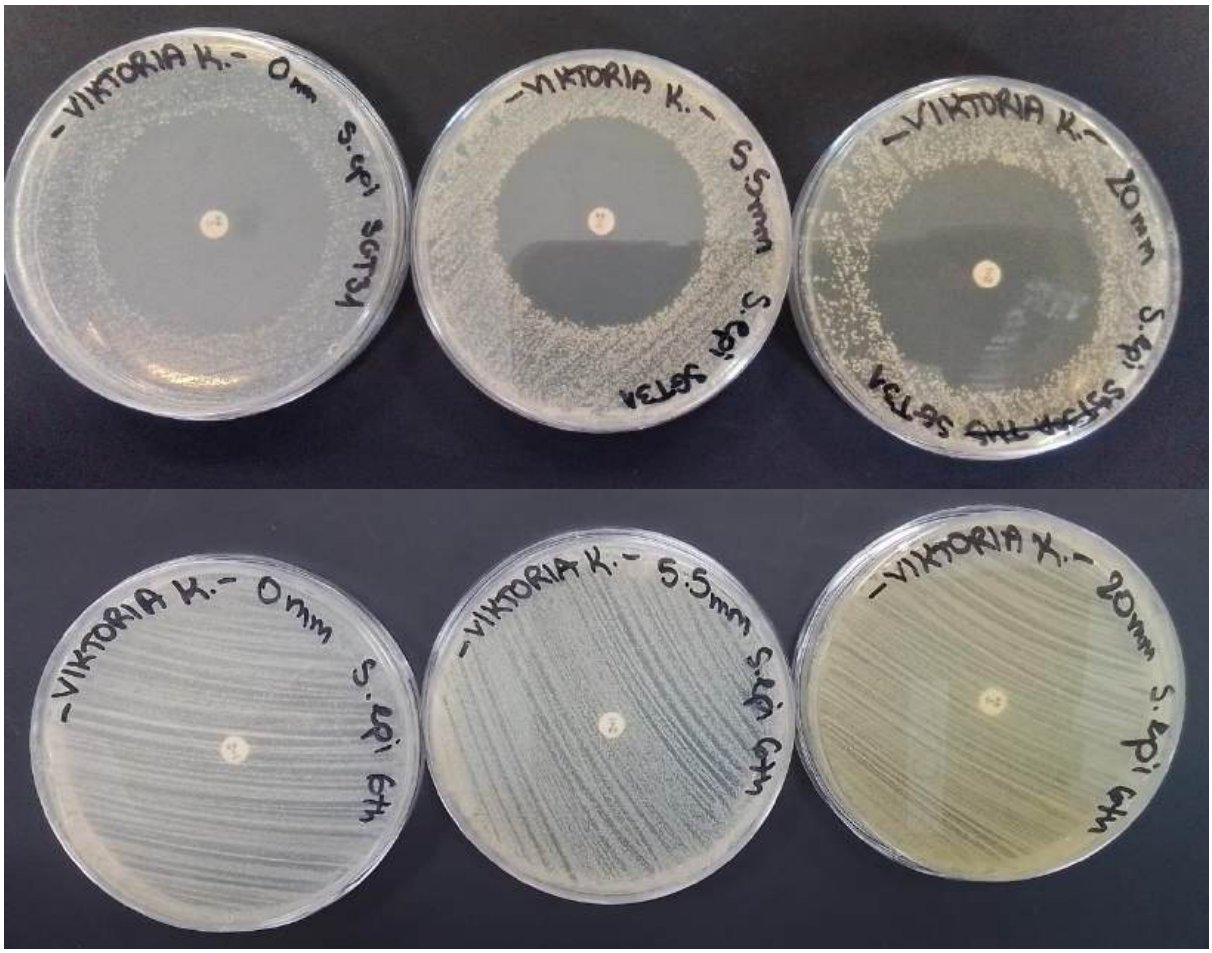

Figure. 4 Pictures showing the complete adaptation (resistance) of *S. epidermidis* strain to Rifampicin after 6 passages at all glucose concentrations.

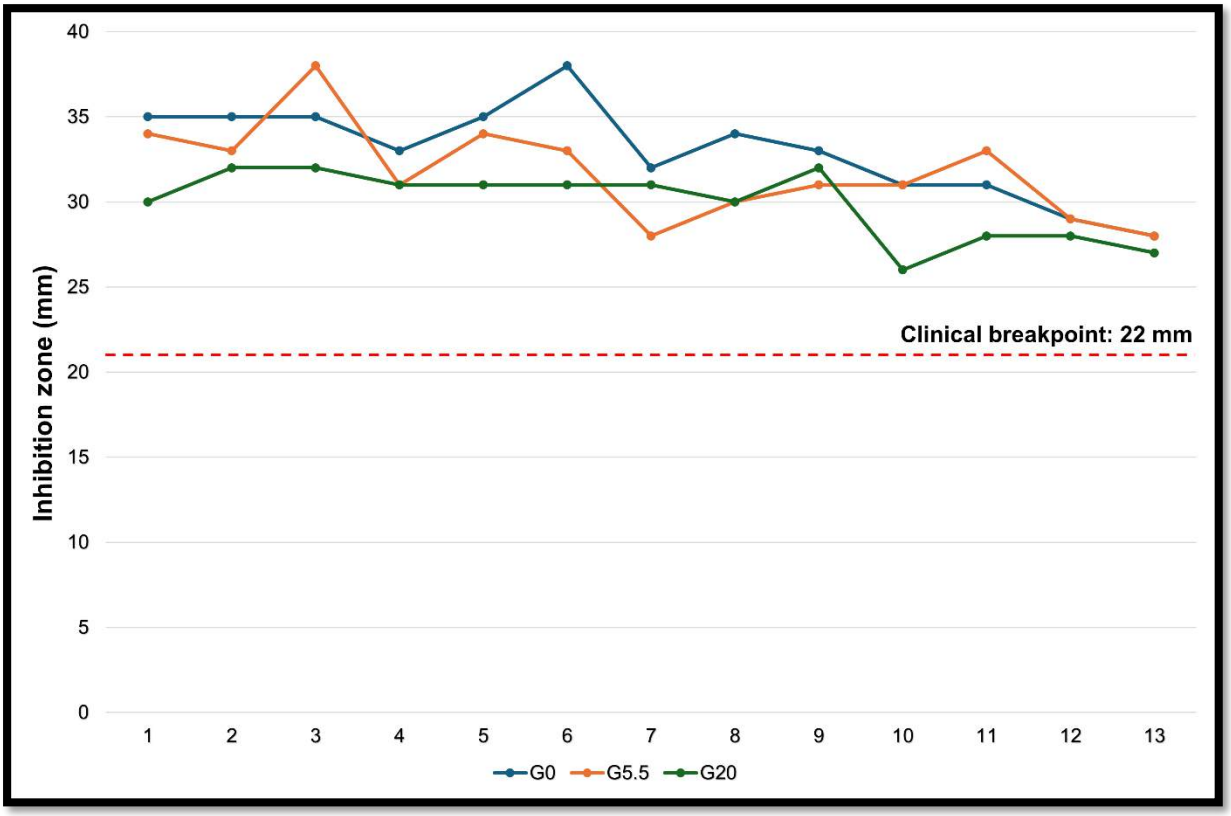

Figure. 5 *S. aureus* adaptation to Clindamycin (2µg) within the 13 passages. X axe showing the inhibition zone sizes and Y axe the number of passages. Blue line shows the adaptation on 0mm glucose (G0); Orange line shows the adaptation on 5.5mm glucose (G5.5); Green line shows the adaptation on 20mm glucose (G20). Red dashed line shows the clinical breakpoint for *S. aureus* on Clindamycin. The graph shows that complete resistance did not occur, however <2-fold decrease was observed at all glucose levels after the 13 passages.

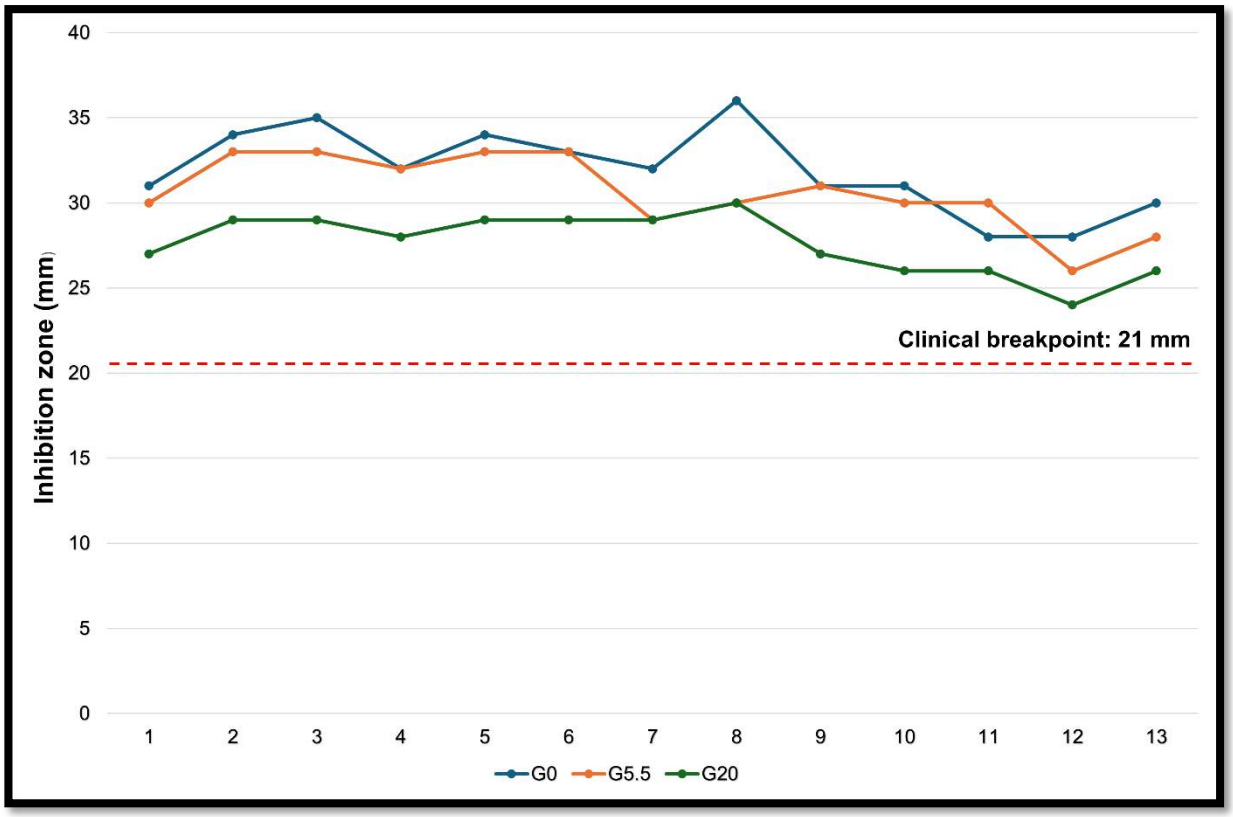

Figure. 6 *S. aureus* adaptation to Erythromycin (15µg) within the 13 passages. X axe showing the inhibition zone sizes and Y axe the number of passages. Blue line shows the adaptation on 0mm glucose (G0); Orange line shows the adaptation on 5.5mm glucose (G5.5); Green line shows the adaptation on 20mm glucose (G20). Red dashed line shows the clinical breakpoint for *S. aureus* on Erythromycin. The graph shows that complete resistance did not occur, however <2-fold decrease was observed at all glucose levels after the 13 passages.

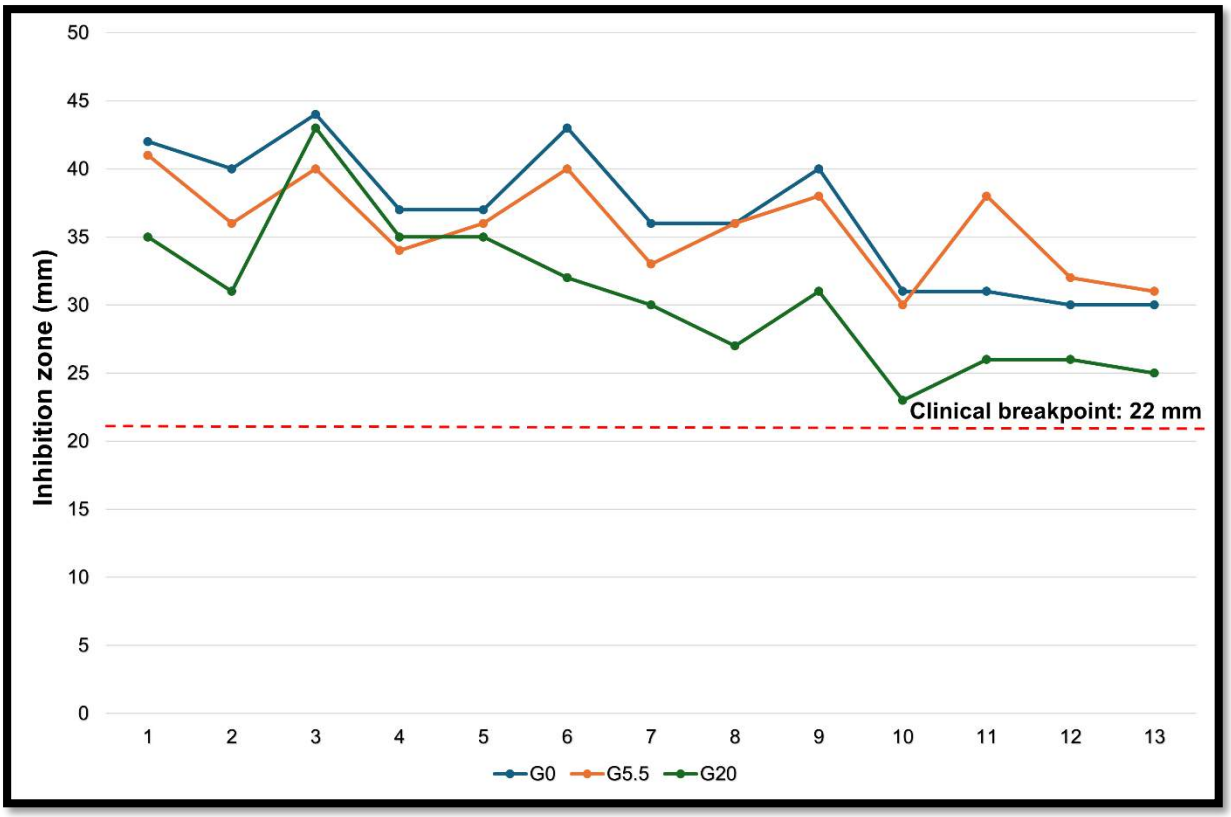

Figure. 7 *S. epidermidis* adaptation to Clindamycin (2µg) within the 13 passages. X axe showing the inhibition zone sizes and Y axe the number of passages. Blue line shows the adaptation on 0mm glucose (G0); Orange line shows the adaptation on 5.5mm glucose (G5.5); Green line shows the adaptation on 20mm glucose (G20). Red dashed line shows the clinical breakpoint for *S. epidermidis* on Clindamycin. The graph shows that complete resistance did not occur, however <2-fold decrease was observed at all glucose levels after the 13 passages.

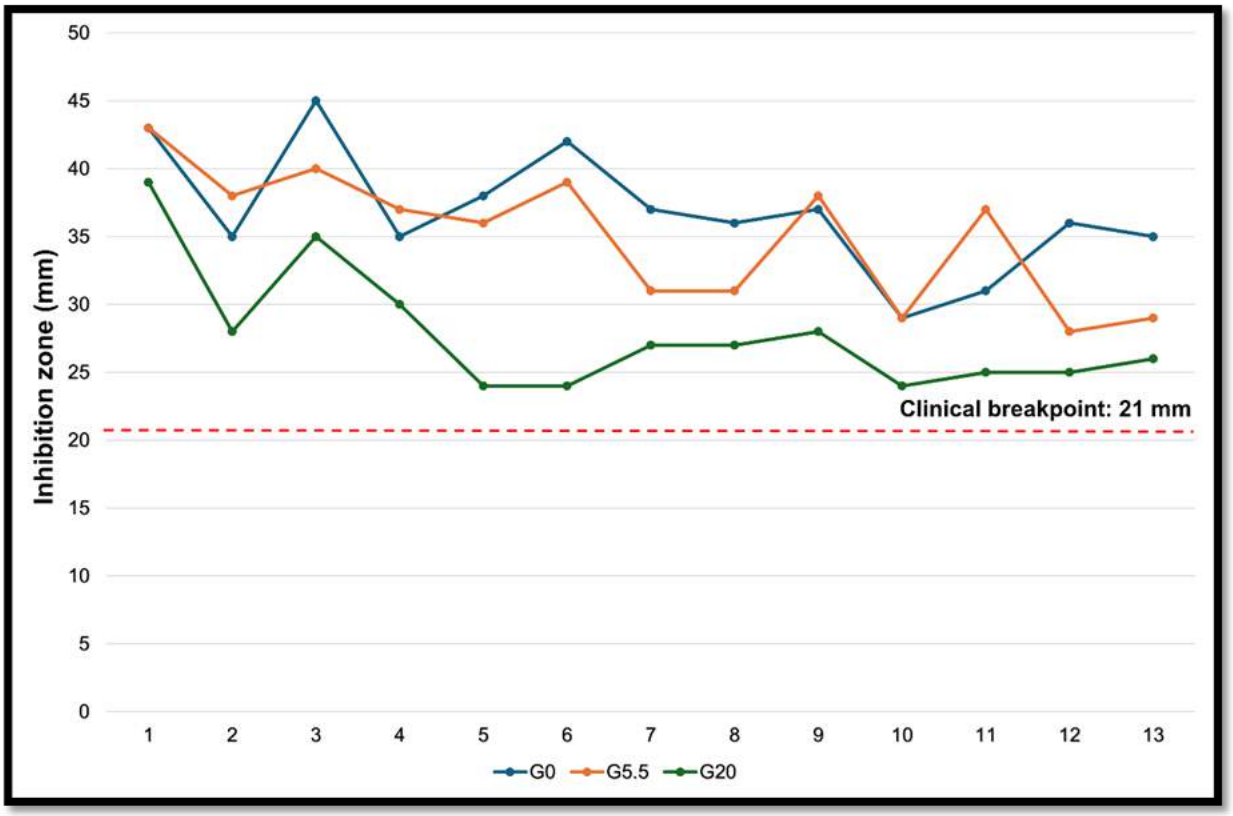

Figure. 8 *S. epidermidis* adaptation to Erythromycin (15µg) within the 13 passages. X axe showing the inhibition zone sizes and Y axe the number of passages. Blue line shows the adaptation on 0mm glucose (G0); Orange line shows the adaptation on 5.5mm glucose (G5.5); Green line shows the adaptation on 20mm glucose (G20). Red dashed line shows the clinical breakpoint for *S. epidermidis* on Erythromycin. The graph shows that complete resistance did not occur, however <2-fold decrease was observed at all glucose levels after the 13 passages.

3.1.2. Resistance emergence after serial adaptation on high glucose levels

The null hypothesis (H0) is that there is no significant difference/correlation between high glucose levels and antibiotic susceptibility. Paired T-tests for each antibiotic and glucose level were performed separately, and the results showed: i) significant difference for both bacteria on Rifampicin at all glucose levels (Figure.9, Figure.10); ii) no significant difference for both bacteria on Clindamycin at all glucose levels (Figure.11, Figure.12); iii) no significant difference for both bacteria on Erythromycin at all glucose levels, except of *S. aureus* at 5.5% glucose (Figure.13, Figure.14). Null hypothesis has been rejected for both bacteria on Rifampicin, therefore there is significant difference/correlation between high glucose and antibiotic susceptibility. However, null hypothesis has been rejected for both bacteria on Clindamycin and Erythromycin, therefore there is no significant difference/correlation between high glucose and antibiotic susceptibility for these antibiotics.

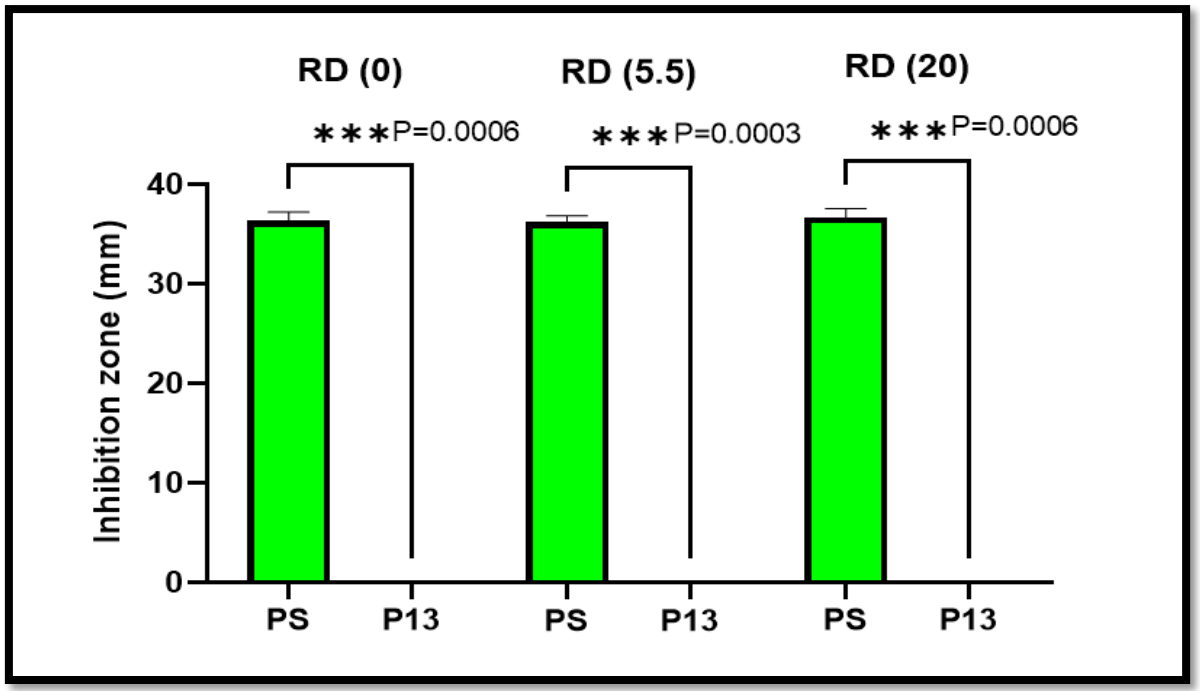

Figure. 9 Column bar graph showing the serial adaptation of *S. aureus* parent strain (PS) and adapted strain (P13) on Rifampicin at three glucose concentrations-0mm (0), 5.5mm (5.5), 20mm (20) after 13 passages. This figure shows a significant difference in antibiotic susceptibility between glucose concentrations (P=0.0006, DF=2, SD=1.528; P=0.0003, DF=2, SD=1.155; P=0.0006, DF=2, SD=1.528), with an overall decrease in susceptibility for the adapted strain.

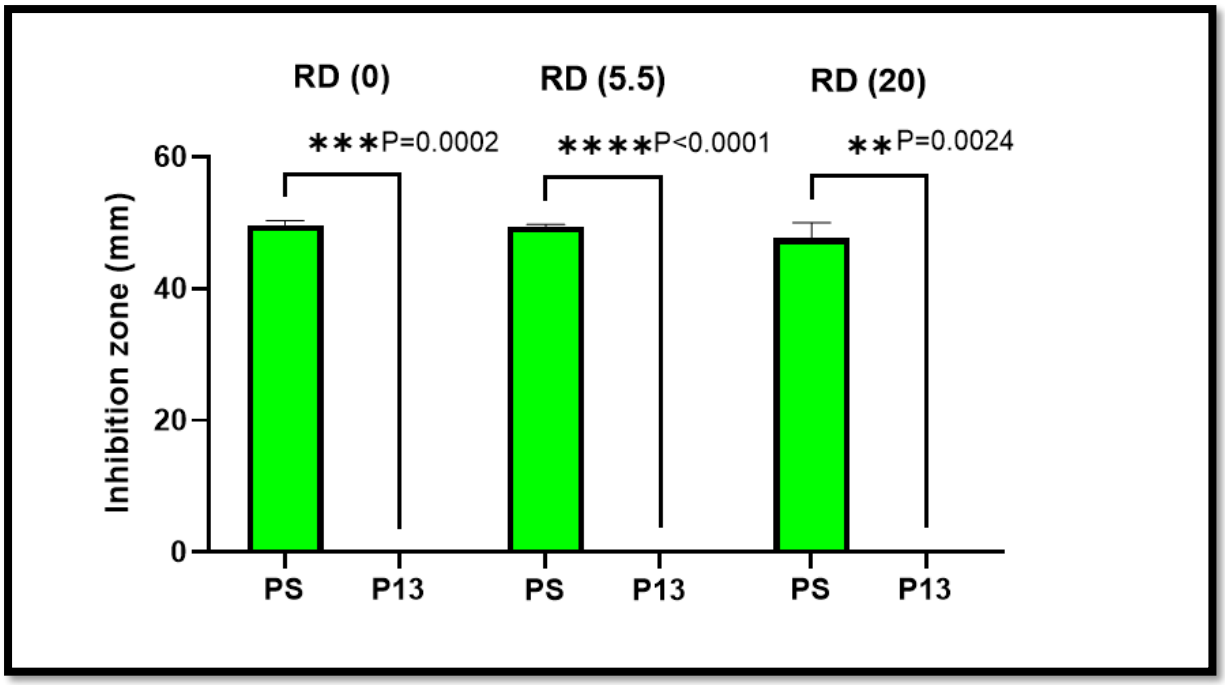

Figure. 10 Column bar graph showing the serial adaptation of *S. epidermidis* parent strain (PS) and adapted strain (P13) on Rifampicin at three glucose concentrations-0mm (0), 5.5mm (5.5), 20mm (20) after 13 passages. This figure shows a significant difference in antibiotic susceptibility between glucose concentrations (P=0.0002, DF=2, SD=1.155; P<0.0001, DF=2, SD= 0.5774; P=0.0024, DF=2, SD=4.041), with an overall decrease in susceptibility for the adapted strain.

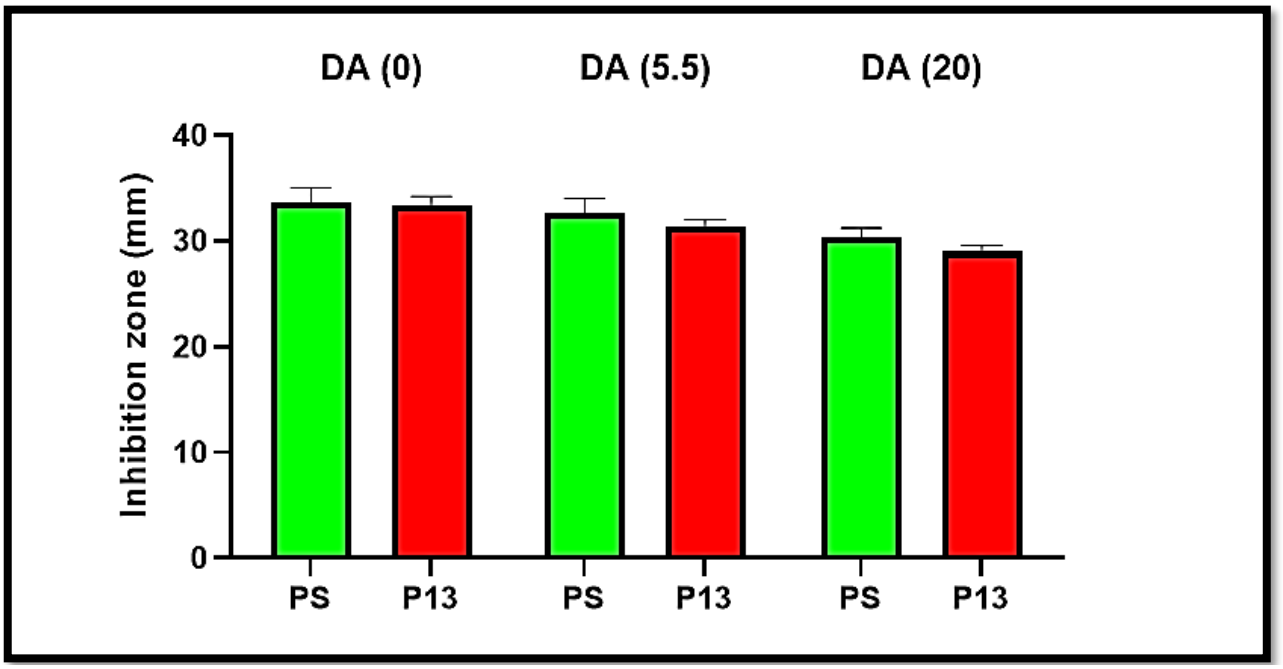

Figure. 11 Column bar graph showing the serial adaptation of *S. aureus* parent strain (PS) and adapted strain (P13) on Clindamycin at three glucose concentrations-0mm (0), 5.5mm (5.5), 20mm (20) after 13 passages. This figure shows no significant difference in antibiotic susceptibility between glucose concentrations (P=0.7418, DF=2, SD=1.528; P=0.1835, DF=2, SD=1.155; P=0.4226, DF=2, SD=2.309), with an overall decrease in susceptibility for the adapted strain.

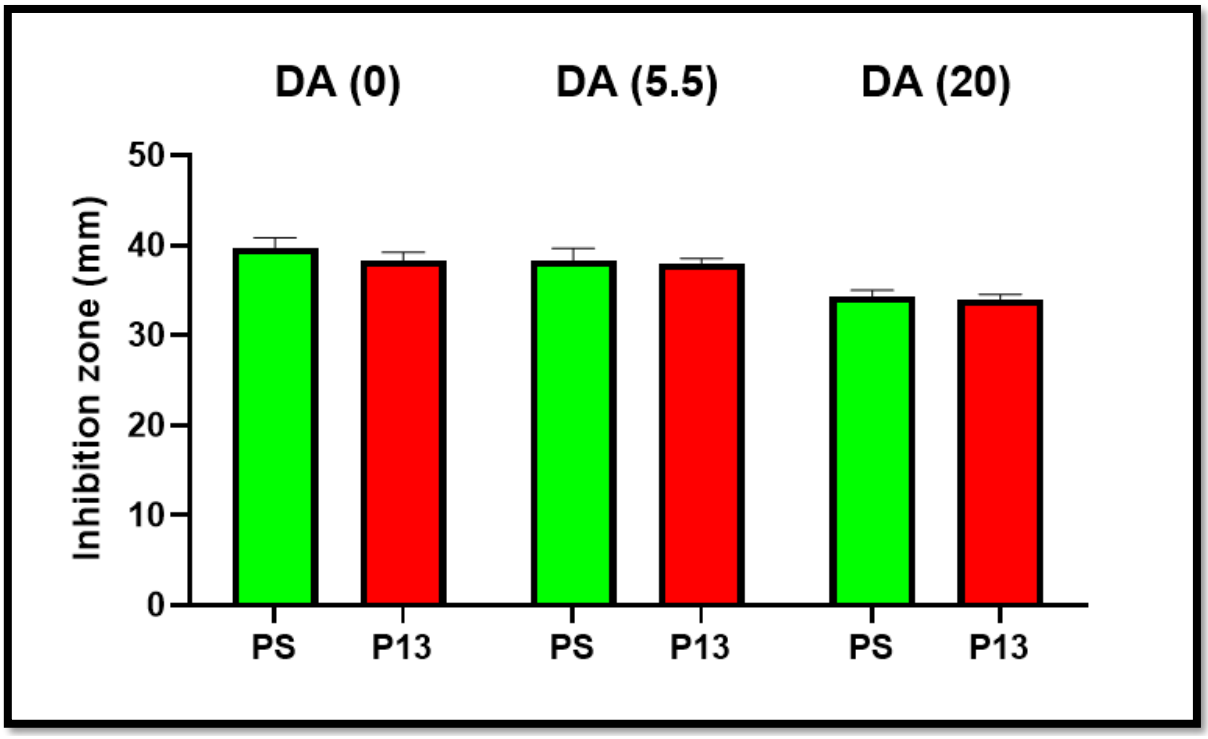

Figure. 12 Column bar graph showing the serial adaptation of *S. epidermidis* parent strain (PS) and adapted strain (P13) on Clindamycin at three glucose concentrations-0mm (0), 5.5mm (5.5), 20mm (20) after 13 passages. This figure shows no significant difference in antibiotic susceptibility between glucose concentrations (P=0.0572, DF=2, SD=0.5774; P=0.7418, DF=2, SD=1.528; P=0.4226, DF=2, SD=0.5774), with an overall decrease in susceptibility for the adapted strain.

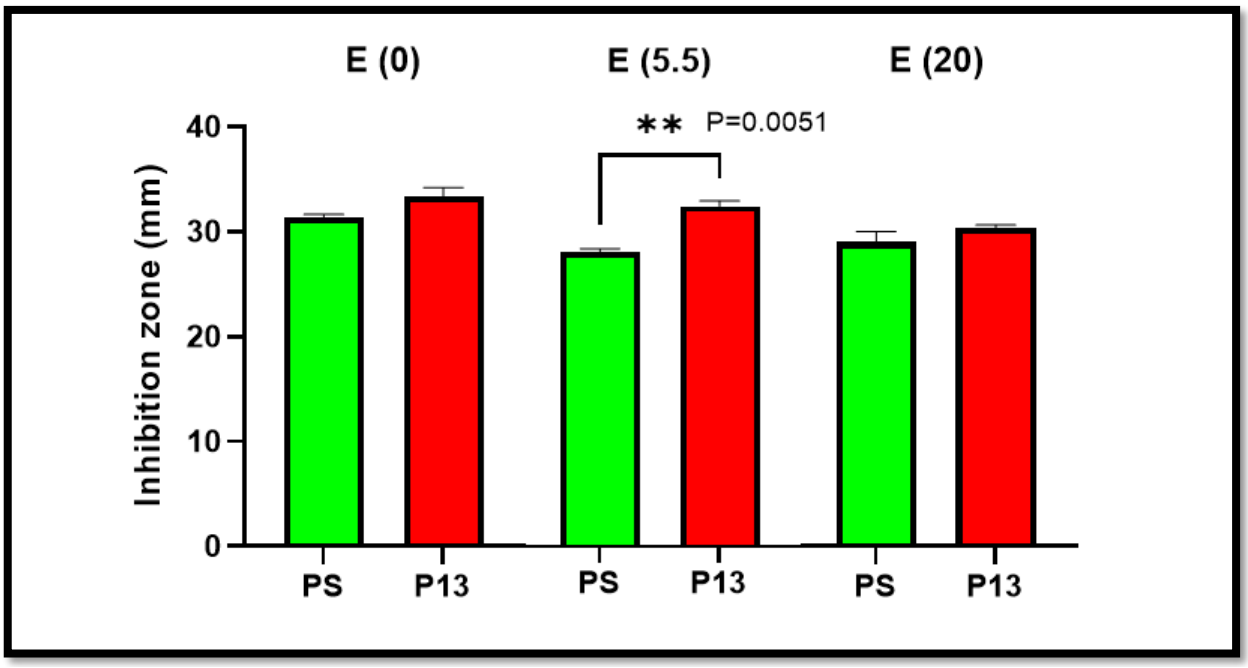

Figure. 13 Column bar graph showing the serial adaptation of *S. aureus* parent strain (PS) and adapted strain (P13) on Erythromycin at three glucose concentrations-0mm (0), 5.5mm (5.5), 20mm (20) after 13 passages. This figure shows no significant difference in antibiotic susceptibility at glucose concentrations of 0mm and 20mm (P=0.0742, DF=2, SD=1.000; P=0.4226, DF=2, SD=2.309), however significant difference at glucose concentration of 5.5mm (P=0.0051, DF=2, SD=0.5774).

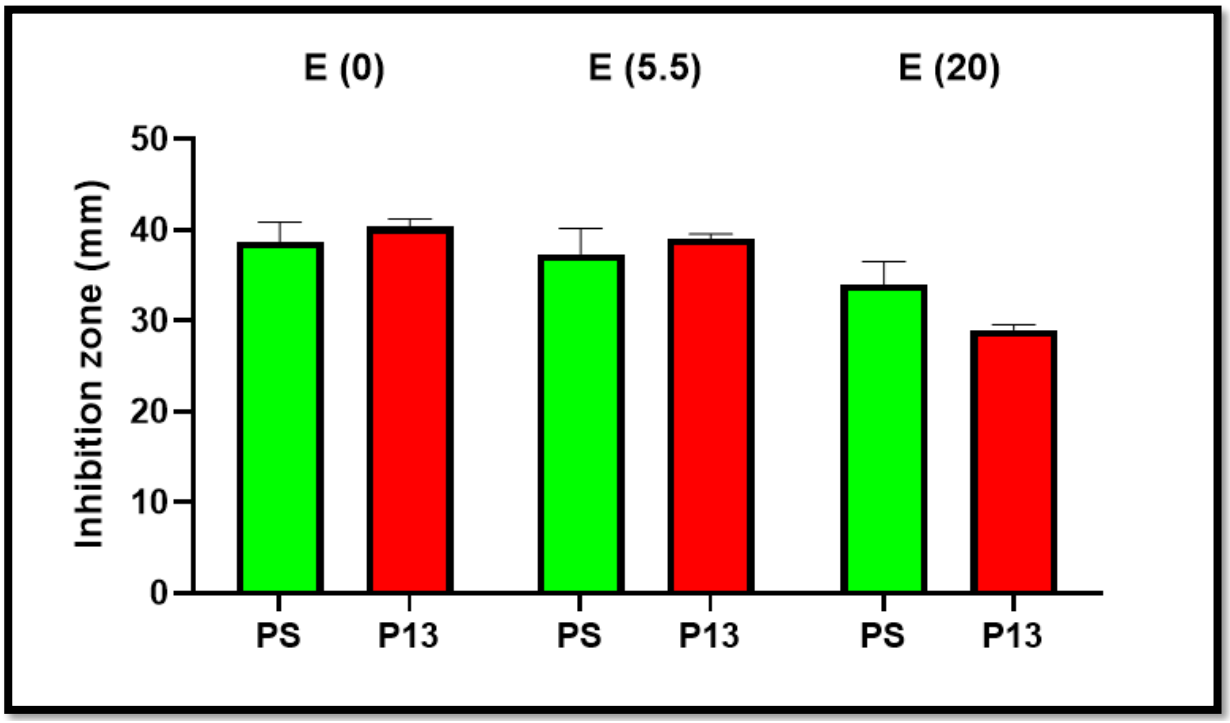

Figure. 14 Column bar graph showing the serial adaptation of *S. epidermidis* parent strain (PS) and adapted strain (P13) on Erythromycin at three glucose concentrations-0mm (0), 5.5mm (5.5), 20mm (20) after 13 passages. This figure shows no significant difference in antibiotic susceptibility between glucose concentrations (P=0.3377, DF=2, SD=2.309; P=0.5492, DF=2, SD=4.041; P=0.1296, DF=2, SD=3.464), with an overall decrease in susceptibility of the adapted strain at glucose concentration of 20mm.

3.2. Relationship between adaptation at high glucose concentration and pH

3.2.1. Adaptation of *S. epidermidis* on Clindamycin, Rifampicin and Erythromycin altered the antibiotic susceptibility at three pH levels

S. epidermidis strains were passaged at three different pH levels on Clindamycin, Rifampicin and Erythromycin. The parent strain, and the adapted strains on Rifampicin at the three glucose concentrations were used. Lower susceptibility on Erythromycin and Clindamycin at lower pH was observed for the parent strain, and the adapted strains at 0mm glucose and at 5.5mm glucose (Figure.15, Figure.16, Figure.17). The same phenomenon was observed for the adapted strain at 20mm glucose on Erythromycin (Figure.18). However, the parent strain showed higher susceptibility on Rifampicin at lower pH (Figure.15). The adapted strains remained resistant to Rifampicin at all pH levels (Figure.16, Figure.17, Figure.18). No cross-resistance was observed.

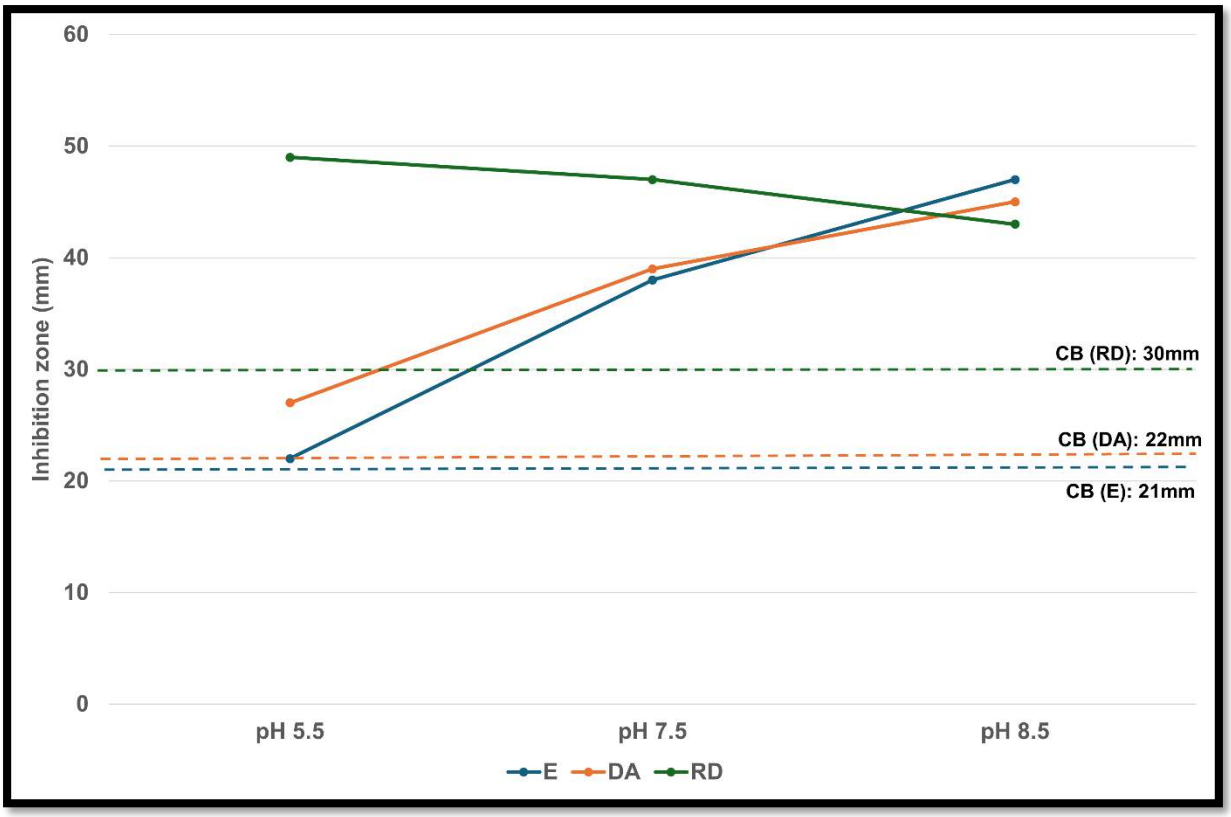

Figure. 15 *S. epidermidis* parent strain's adaptation on Erythromycin (15µg), Clindamycin (2µg) and Rifampicin (5µg) at pH 5.5, pH 7.5, and pH 8.5. X axe showing the inhibition zone sizes and Y axe the pH levels. Blue line shows the adaptation on Erythromycin; Orange line shows the adaptation on Clindamycin; Green line shows the adaptation on Rifampicin. Green, orange, and blue dashed line show the clinical breakpoints for *S. epidermidis* on Erythromycin, Clindamycin, and Rifampicin, respectively. The graph shows lower susceptibility at lower pH on Clindamycin and Erythromycin, and lower susceptibility at higher pH on Rifampicin.

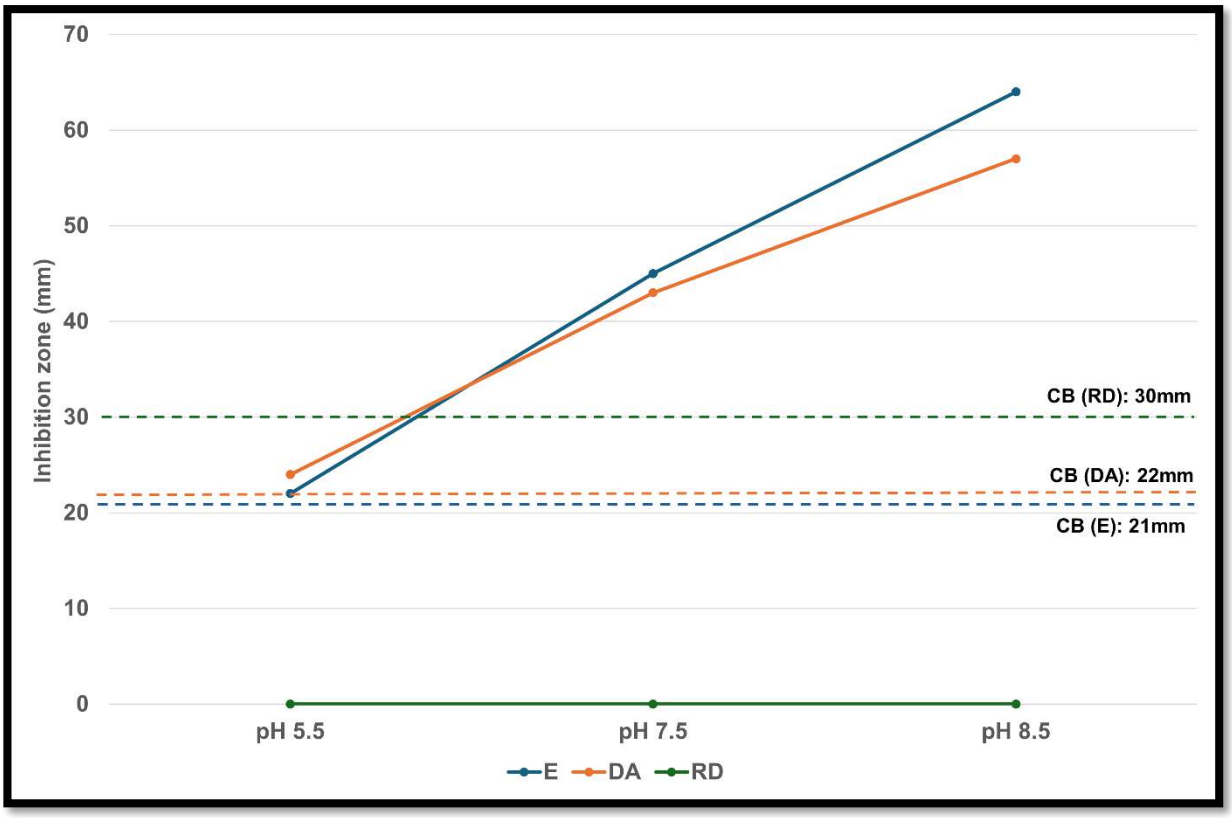

Figure. 16 *S. epidermidis* adapted strain's (SE0) adaptation on Erythromycin (15µg), Clindamycin (2µg) and Rifampicin (5µg) at pH 5.5, pH 7.5, and pH 8.5. X axe showing the inhibition zone sizes and Y axe the pH levels. Blue line shows the adaptation on Erythromycin; Orange line shows the adaptation on Clindamycin; Green line shows the adaptation on Rifampicin. Green, orange, and blue dashed line show the clinical breakpoints for *S. epidermidis* on Erythromycin, Clindamycin, and Rifampicin, respectively. The graph shows lower susceptibility at lower pH on Clindamycin and Erythromycin, and complete resistance remained constant at all pH levels on Rifampicin.

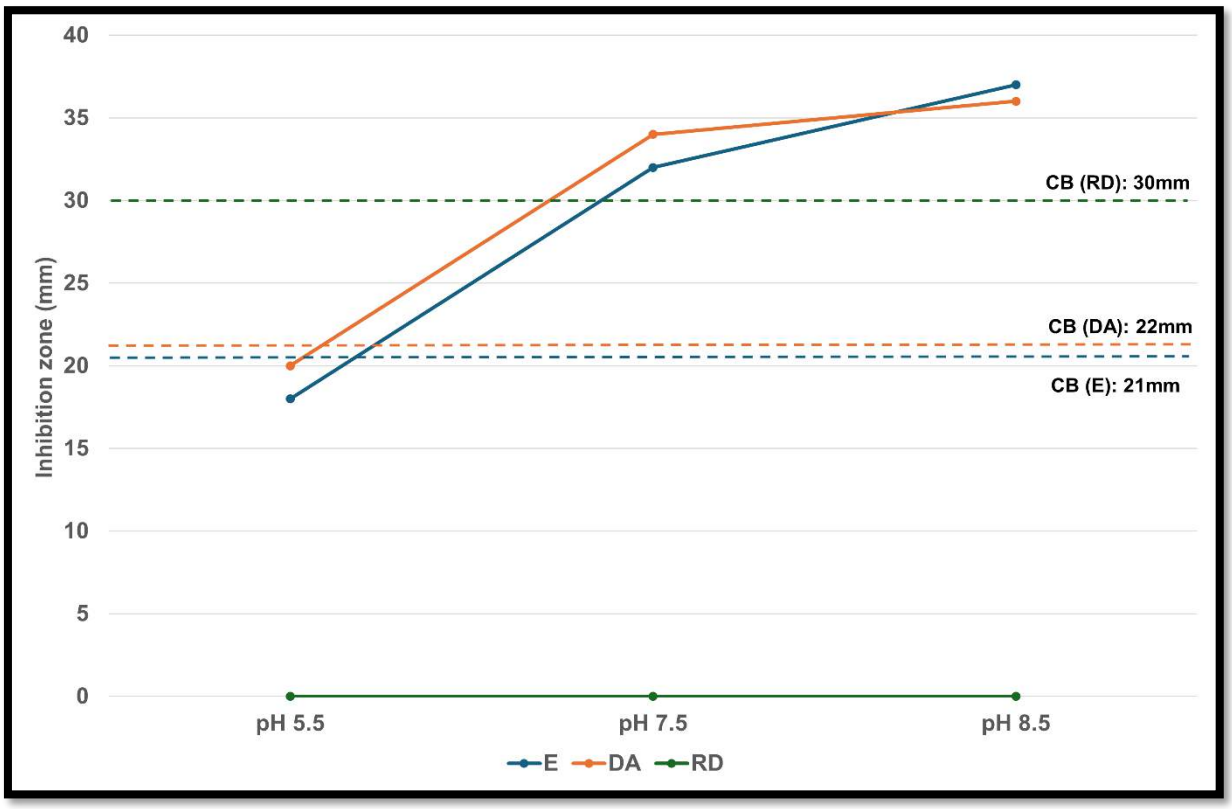

Figure. 17 *S. epidermidis* adapted strain's (SE5.5) adaptation on Erythromycin (15µg), Clindamycin (2µg) and Rifampicin (5µg) at pH 5.5, pH 7.5, and pH 8.5. X axe showing the inhibition zone sizes and Y axe the pH levels. Blue line shows the adaptation on Erythromycin; Orange line shows the adaptation on Clindamycin; Green line shows the adaptation on Rifampicin. Green, orange, and blue dashed line show the clinical breakpoints for *S. epidermidis* on Erythromycin, Clindamycin, and Rifampicin, respectively. The graph shows lower susceptibility at lower pH on Clindamycin and Erythromycin, and complete resistance remained constant at all pH levels on Rifampicin.

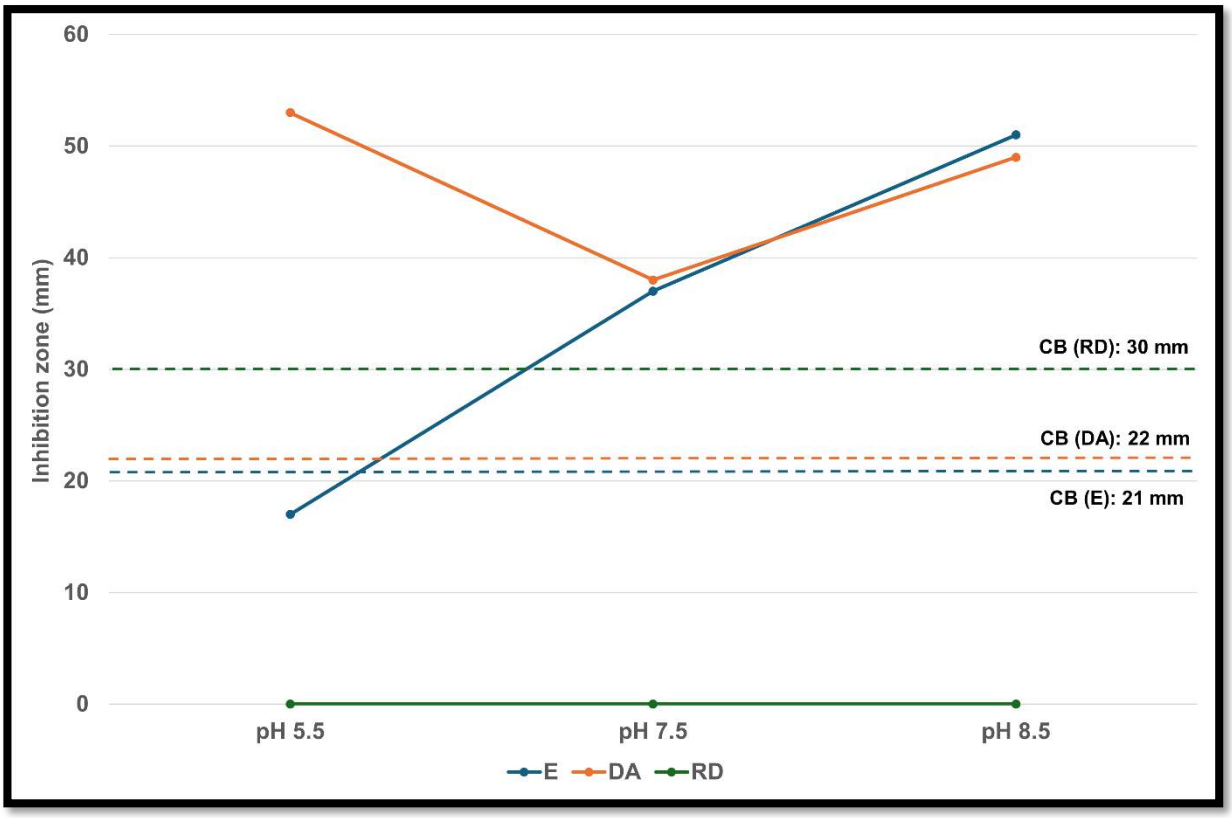

Figure. 18 *S. epidermidis* adapted strain's (SE20) adaptation on Erythromycin (15µg), Clindamycin (2µg) and Rifampicin (5µg) at pH 5.5, pH 7.5, and pH 8.5. X axe showing the inhibition zone sizes and Y axe the pH levels. Blue line shows the adaptation on Erythromycin; Orange line shows the adaptation on Clindamycin; Green line shows the adaptation on Rifampicin. Green, orange, and blue dashed line show the clinical breakpoints for *S. epidermidis* on Erythromycin, Clindamycin, and Rifampicin, respectively. The graph shows lower susceptibility at lower pH on Erythromycin, lower susceptibility at higher pH on Clindamycin, and complete resistance remained constant at all pH levels on Rifampicin.

3.2.2. Comparison of antibiotic susceptibility at three pH levels between parent strain and adapted strains

The null hypothesis (H0) is that there is no significant difference/correlation between high glucose adapted strain's antibiotic susceptibility and pH. Paired T-tests for each antibiotic, glucose concentration and pH level were performed separately, and the results showed: i) significant difference for all strains on Rifampicin, Clindamycin and Erythromycin at all pH levels (Figure.19-Figure.27); ii) no significant difference for the adapted strain at 0 mm glucose on Erythromycin at pH 5.5 (Figure.21); iii) no significant difference for the adapted strain at 20 mm glucose on Clindamycin at pH 7.5 and pH 8.5, and Erythromycin at pH 7.5 (Figure.25, Figure.27). Null hypothesis has been rejected for all adapted strains on Rifampicin, and most of the adapted strains on Erythromycin and Clindamycin. Therefore, there is significant difference/correlation between high glucose adapted strain's antibiotic susceptibility and pH.

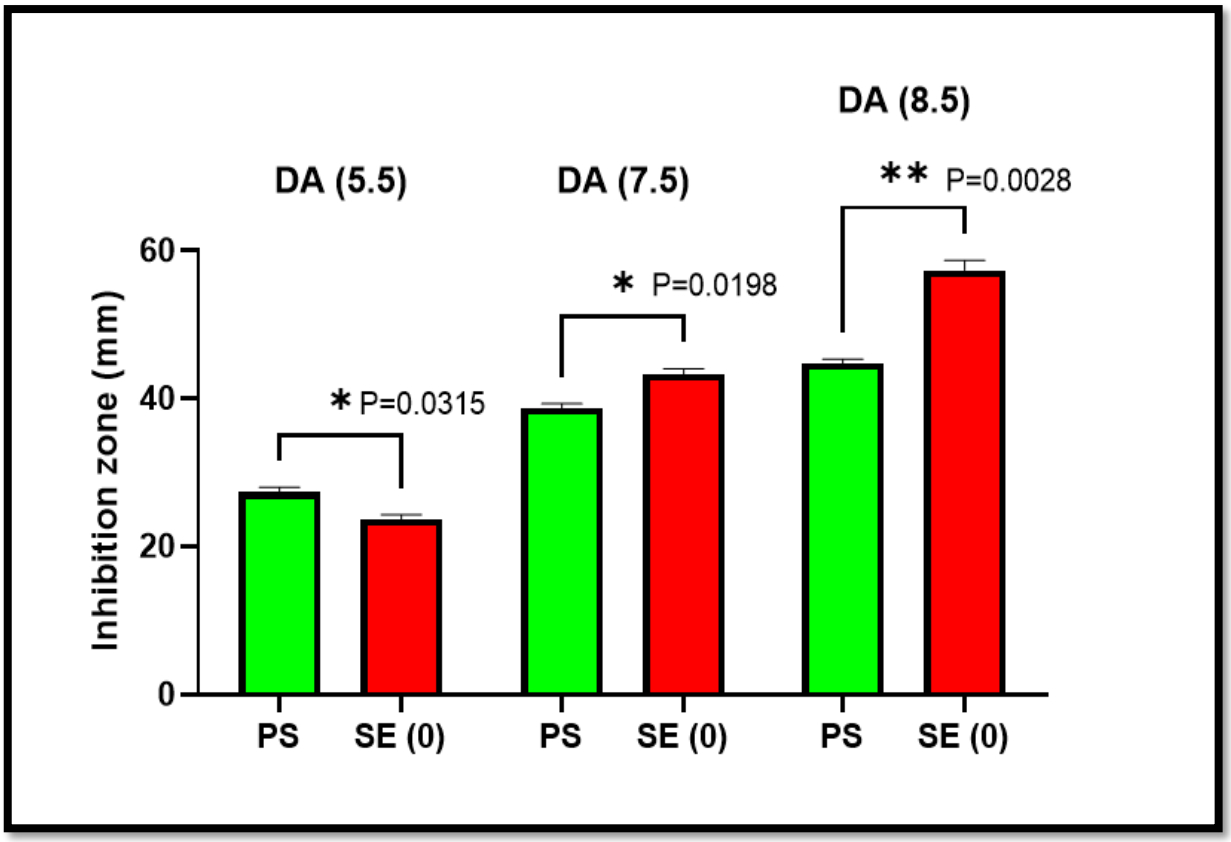

Figure. 19 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE0) on Clindamycin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at all pH levels (P=0.0315, DF=2, SD=1.155; P=0.0198, DF=2, SD=1.155; P=0.0028, DF=2, SD=1.155).

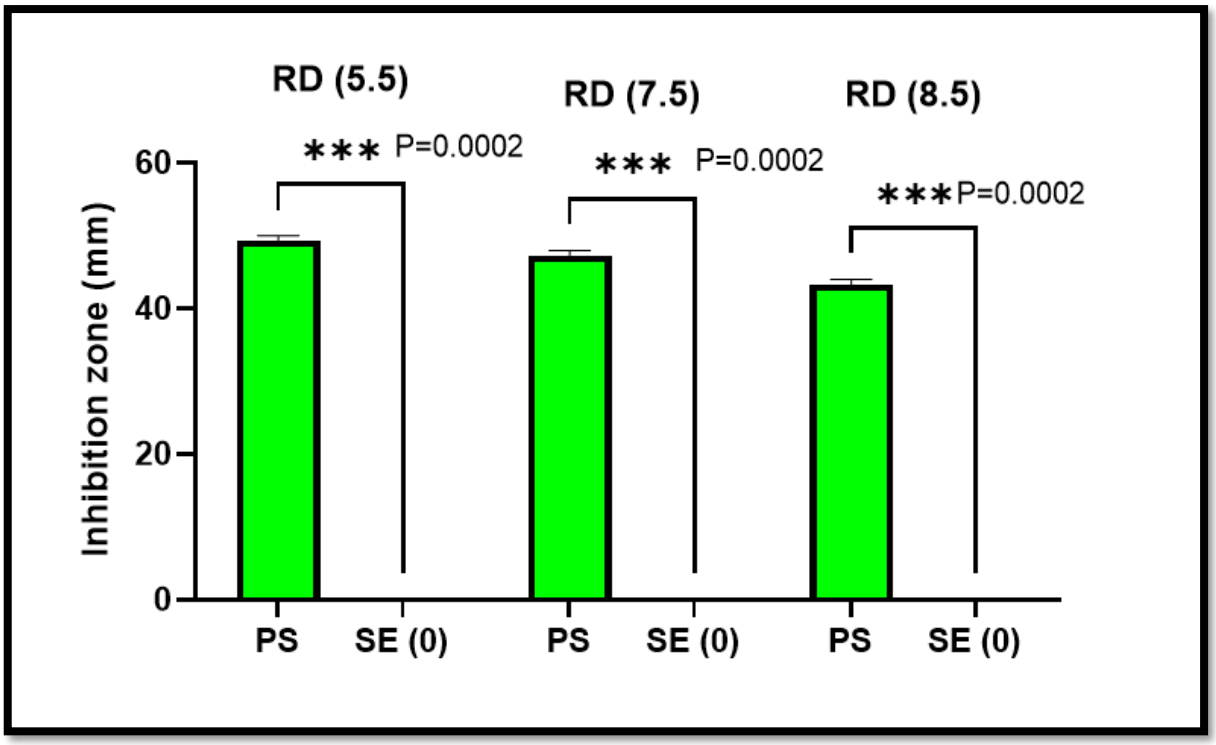

Figure. 20 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE0) on Rifampicin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at all pH levels (P=0.0002, DF=2, SD=1.155 for all three).

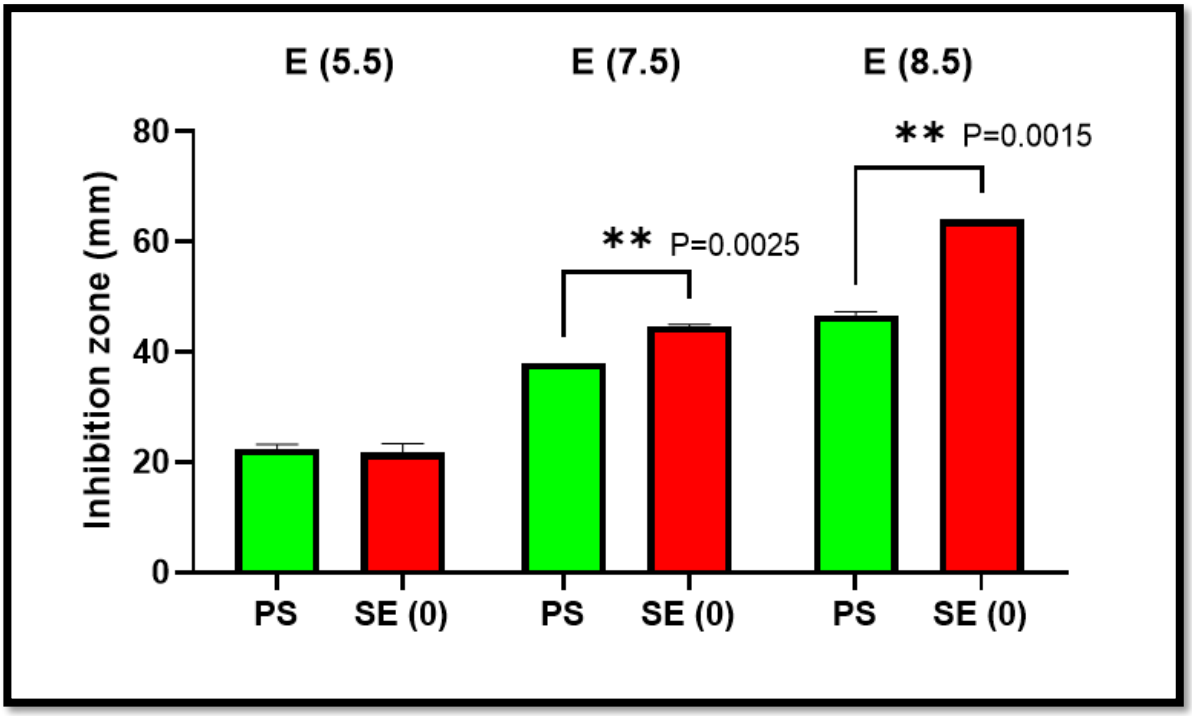

Figure. 21 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE0) on Erythromycin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at pH levels of 7.5 and 8.5 (P=0.0025, DF=2, SD=0.5774; P=0.0015, DF=2, SD=1.155). No significance was observed at pH 5.5 (P=0.5286, DF=2, SD=1.528).

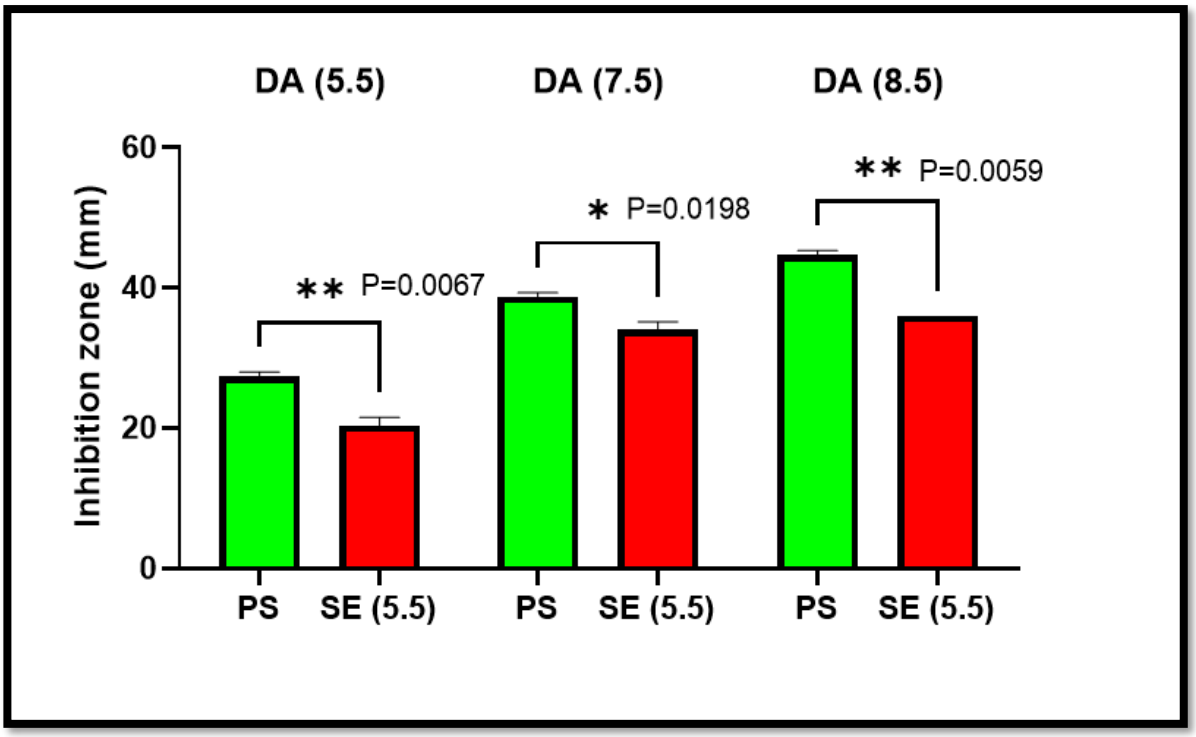

Figure. 22 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE5.5) on Clindamycin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at all pH levels (P=0.0067, DF=2, SD=1.000; P=0.0198, DF=2, SD=1.155; P=0.0059, DF=2, SD=1.155).

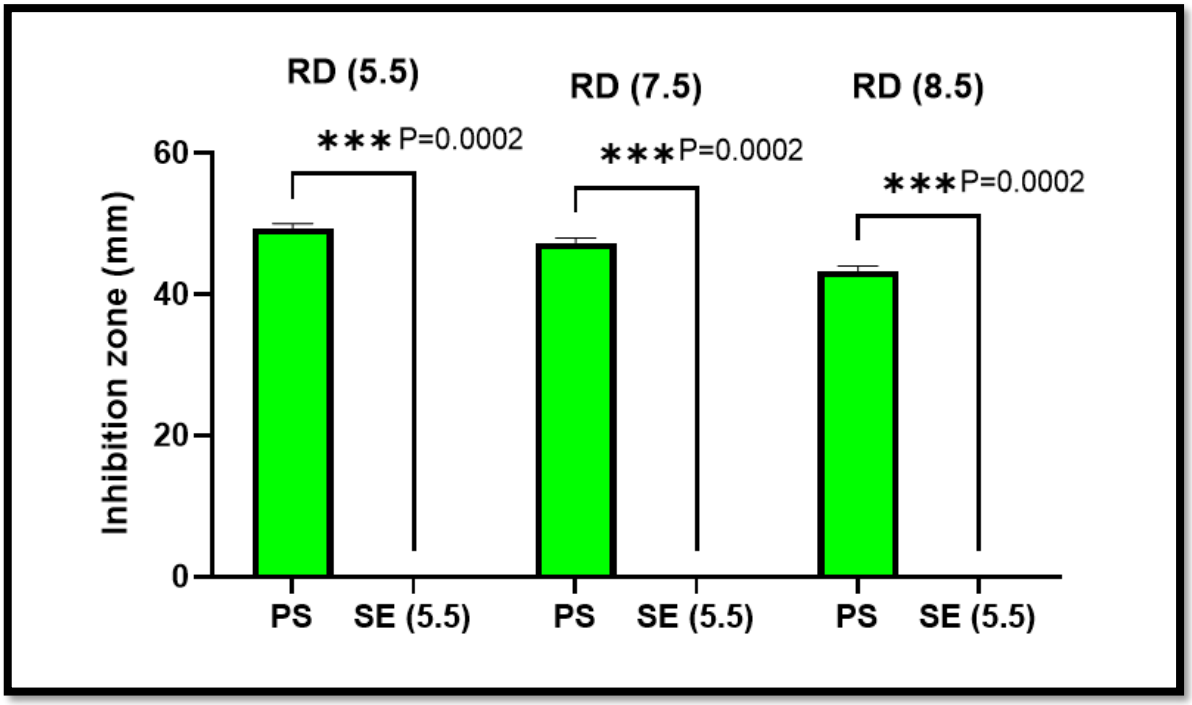

Figure. 23 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE5.5) on Rifampicin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at all pH levels (P=0.0002, DF=2, SD=1.155 for all three).

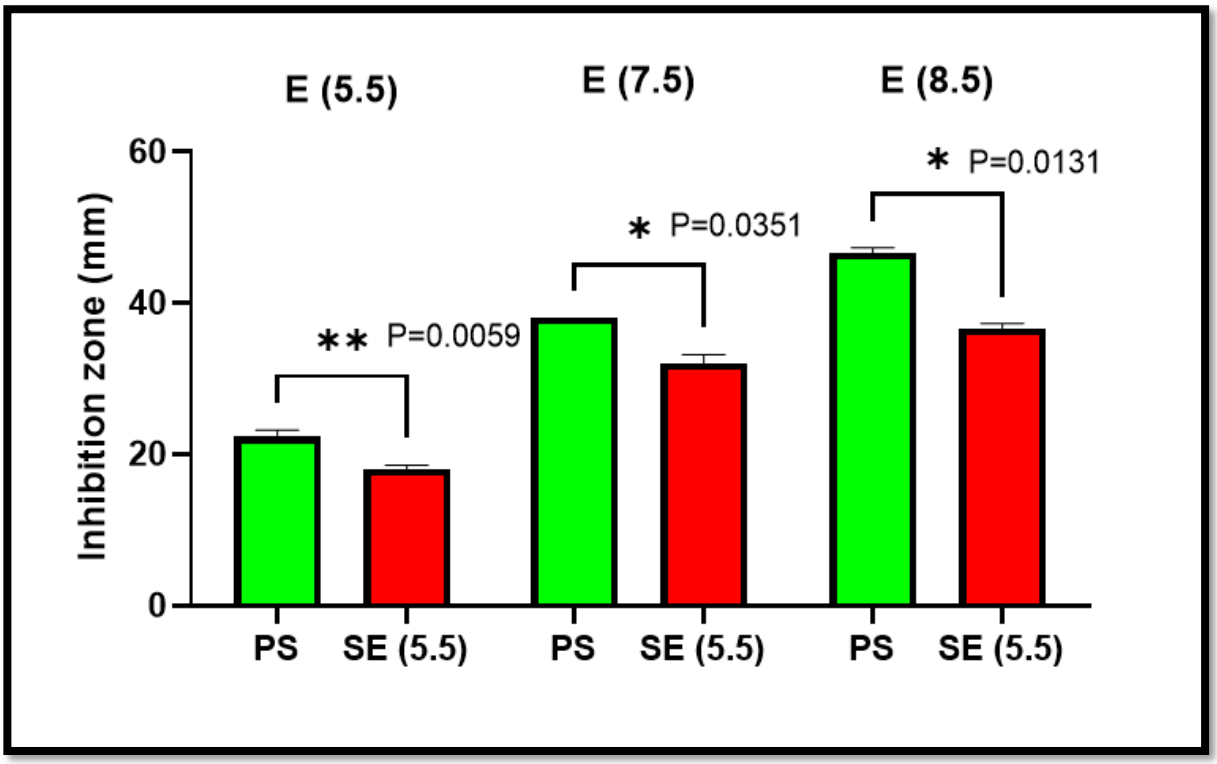

Figure. 24 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE5.5) on Erythromycin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at all pH levels (P=0.0059, DF=2, SD=0.5774, P=0.0351, DF=2, SD=2.000, P=0.0131, DF=2, SD=2.000).

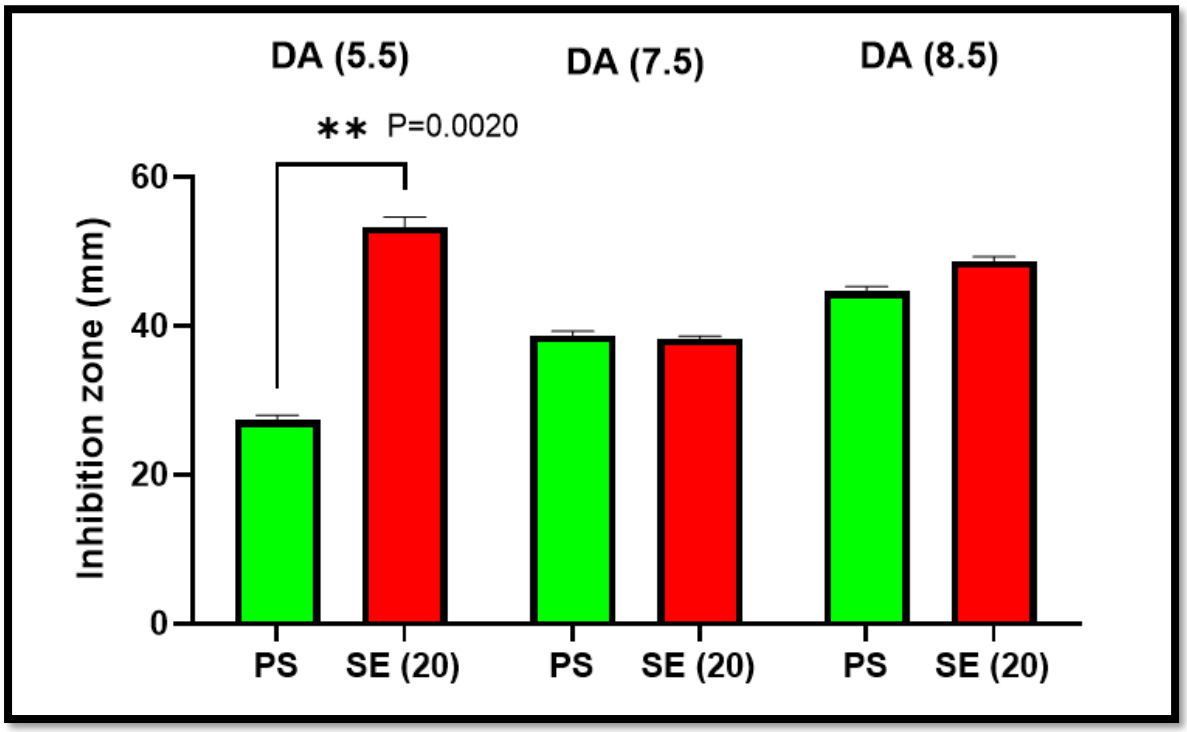

Figure. 25 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE20) on Clindamycin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at pH 5.5 (P=0.0020, DF=2, SD=2.000). No significance was observed at pH 7.5 and pH 8.5 (P=0.4226, DF=2, SD=0.5774; P=0.0742, DF=2, SD=2.000).

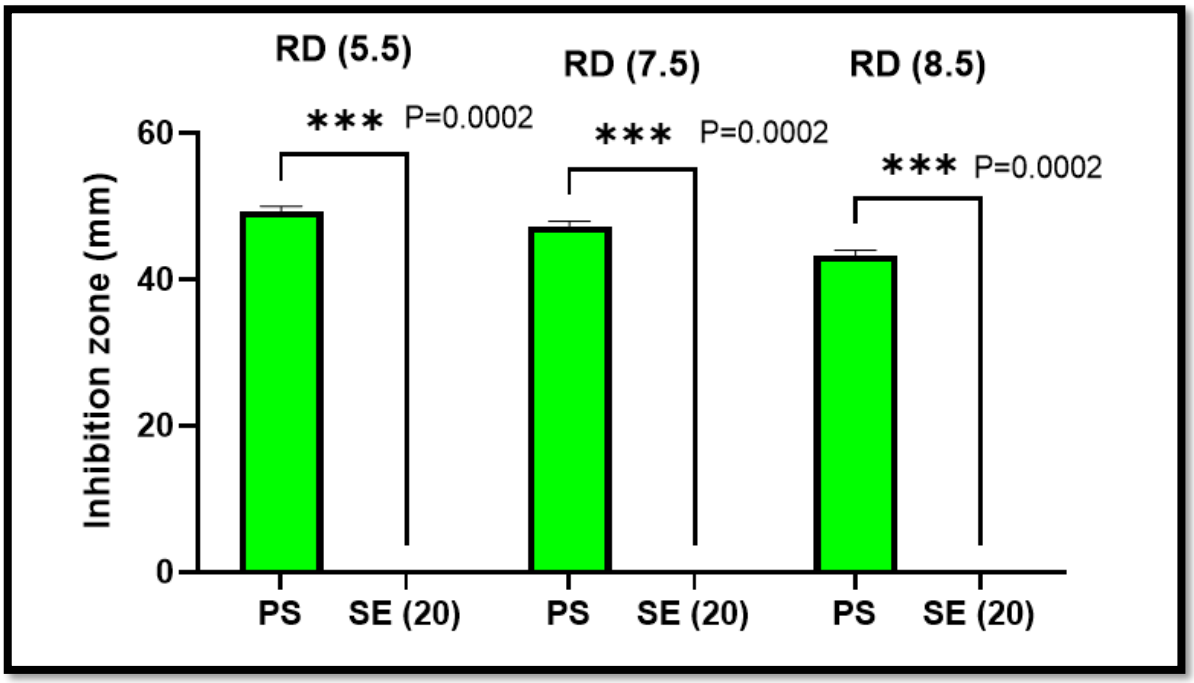

Figure. 26 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE20) on Rifampicin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at all pH levels (P=0.0002, DF=2, SD=1.155 for all three).

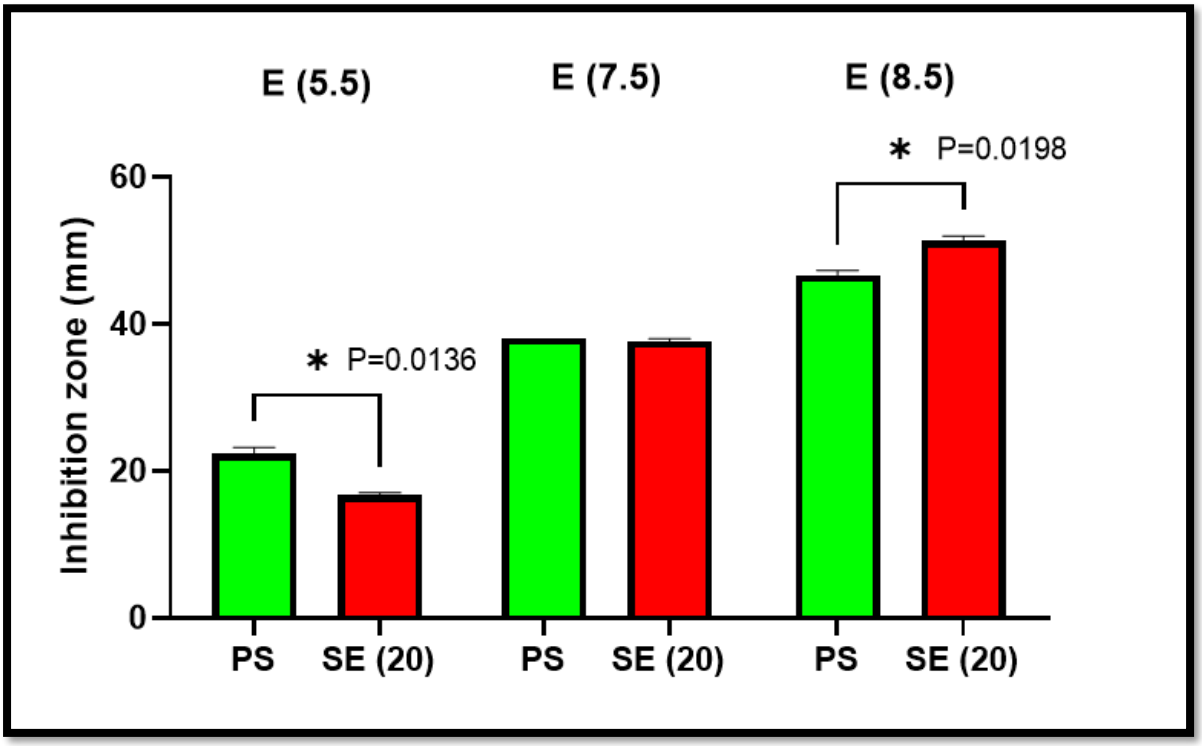

Figure. 27 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE20) on Erythromycin at three pH levels- 5.5, 7.5, and 8.5. This figure shows significant difference in antibiotic susceptibility at pH 5.5 and pH 8.5 (P=0.0136, DF=2, SD=1.155; P=0.0198, DF=2, SD=1.155). No significance was observed at pH 7.5 (P=0.4226, DF=2, SD=0.5774).

3.3. Relationship between adaptation at high glucose concentration and temperature

3.3.1. Adaptation of *S. epidermidis* on Clindamycin, Rifampicin and Erythromycin altered the antibiotic susceptibility at three incubation temperatures

S. epidermidis strains were passaged and incubated at three different temperatures on Clindamycin, Rifampicin and Erythromycin. The parent strain, and the adapted strains on Rifampicin at the three glucose concentrations were used. Lower

susceptibility on Erythromycin, Clindamycin, and Rifampicin at lower temperature was observed for the parent strain (Figure. 28). The same phenomenon was observed on Erythromycin and Clindamycin for the adapted strains at 5.5mm and 20mm glucose (Figure. 29, Figure. 30). The adapted strains remained resistant to Rifampicin at all temperatures (Figure. 29, Figure. 30). Minimal growth was observed at all temperatures for the adapted strain at 0mm glucose, therefore there were no adequate results for this control strain. No cross-resistance was observed.

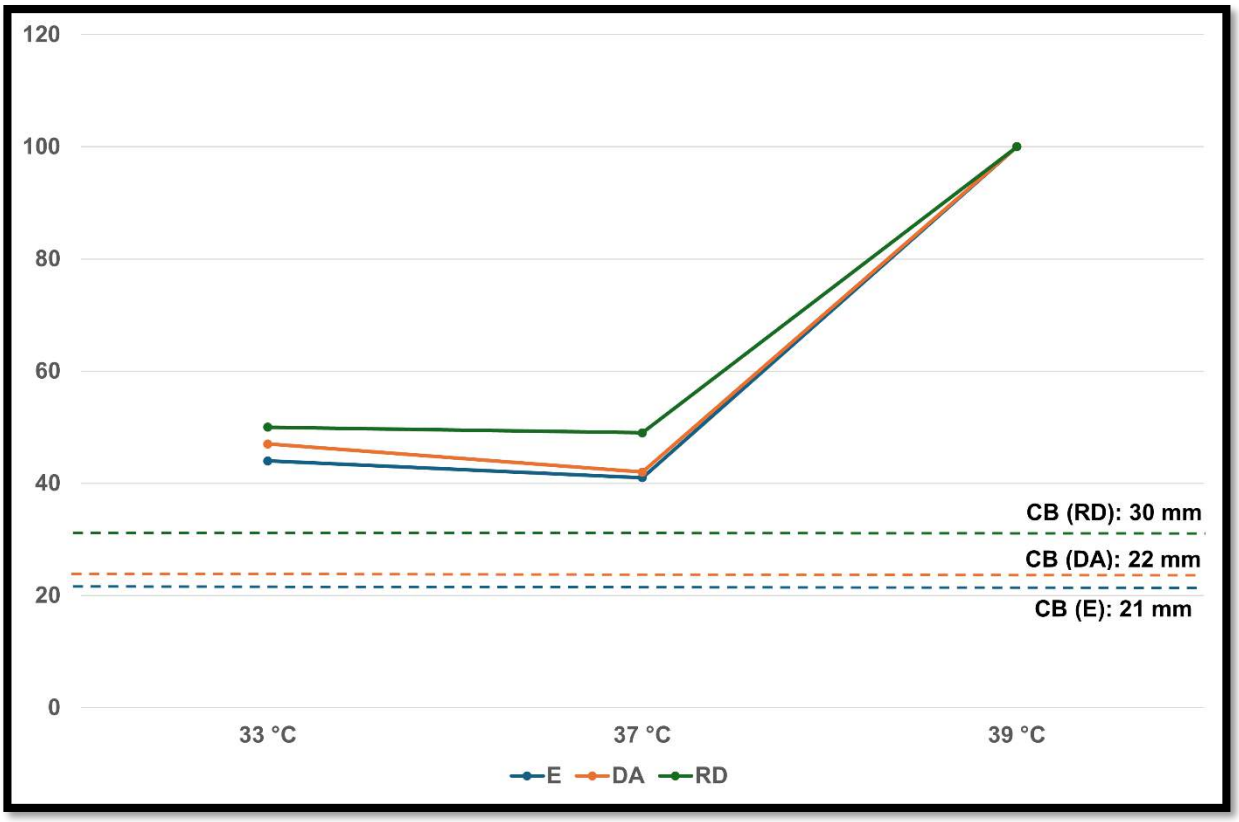

Figure. 28 *S. epidermidis* parent strain's adaptation on Erythromycin (15µg), Clindamycin (2µg) and Rifampicin (5µg) incubated at 33 °C, 37 °C, and 39 °C. X axe showing the inhibition zone sizes and Y axe the temperatures. Blue line shows the adaptation on Erythromycin; Orange line shows the adaptation on Clindamycin; Green line shows the adaptation on Rifampicin. Green, orange, and blue dashed line show the clinical breakpoints for *S. epidermidis* on Erythromycin, Clindamycin, and Rifampicin, respectively. The graph shows lower susceptibility at lower temperature on all three antibiotics.

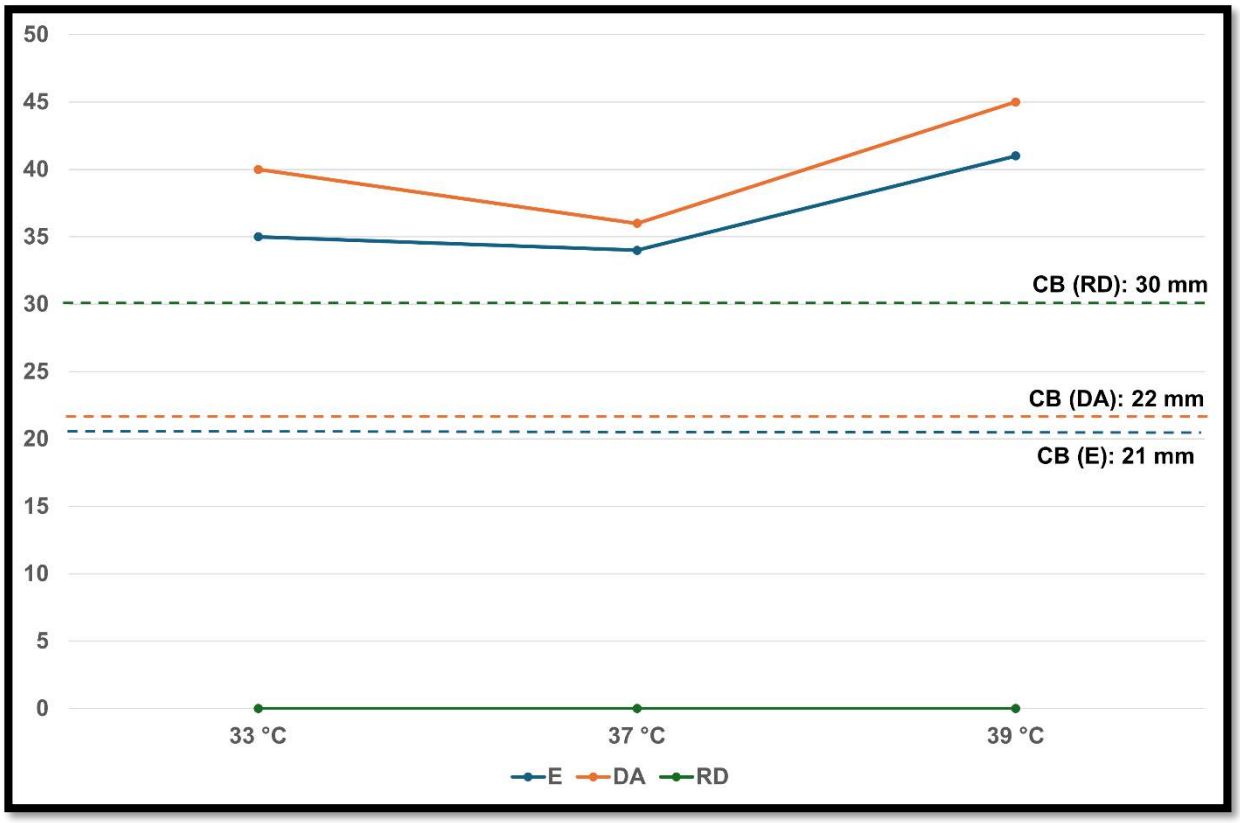

Figure. 29 *S. epidermidis* adapted strain's (SE5.5) adaptation on Erythromycin (15µg), Clindamycin (2µg) and Rifampicin (5µg) incubated at 33 °C, 37 °C, and 39 °C. X axe showing the inhibition zone sizes and Y axe the temperatures. Blue line shows the adaptation on Erythromycin; Orange line shows the adaptation on Clindamycin; Green line shows the adaptation on Rifampicin. Green, orange, and blue dashed line show the clinical breakpoints for *S. epidermidis* on Erythromycin, Clindamycin, and Rifampicin, respectively. The graph shows lower susceptibility at lower temperature on Erythromycin and Clindamycin, and complete resistance remained constant at all temperatures on Rifampicin.

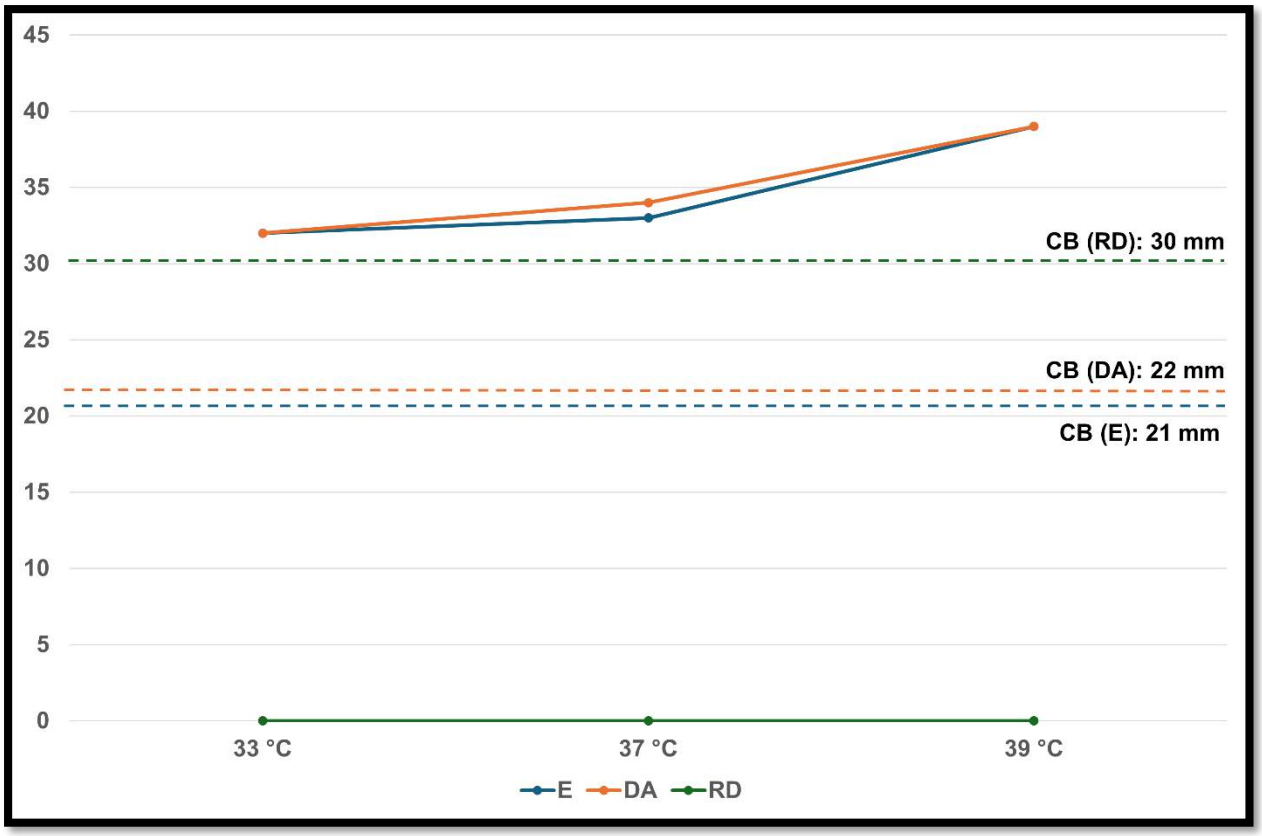

Figure. 30 *S. epidermidis* adapted strain's (SE20) adaptation on Erythromycin (15µg), Clindamycin (2µg) and Rifampicin (5µg) incubated at 33 °C, 37 °C, and 39 °C. X axe showing the inhibition zone sizes and Y axe the temperatures. Blue line shows the adaptation on Erythromycin; Orange line shows the adaptation on Clindamycin; Green line shows the adaptation on Rifampicin. Green, orange, and blue dashed line show the clinical breakpoints for *S. epidermidis* on Erythromycin, Clindamycin, and Rifampicin, respectively. The graph shows lower susceptibility at lower temperature on Erythromycin and Clindamycin, and complete resistance remained constant at all temperatures on Rifampicin.

3.3.2. Comparison of antibiotic susceptibility at three temperatures between parent strain and adapted strains

The null hypothesis (H0) is that there is no significant difference/correlation between high glucose adapted strain's antibiotic susceptibility and temperature. Paired T-tests for each antibiotic, glucose concentration and temperature were performed separately, and the results showed significant difference for both strains (SE5.5, SE20) on Rifampicin, Clindamycin and Erythromycin at all temperatures (Figure.31-Figure.36). Null hypothesis has been rejected for both adapted strains on Rifampicin,

Erythromycin and Clindamycin. Therefore, there is significant difference/correlation between high glucose adapted strain's antibiotic susceptibility and temperature.

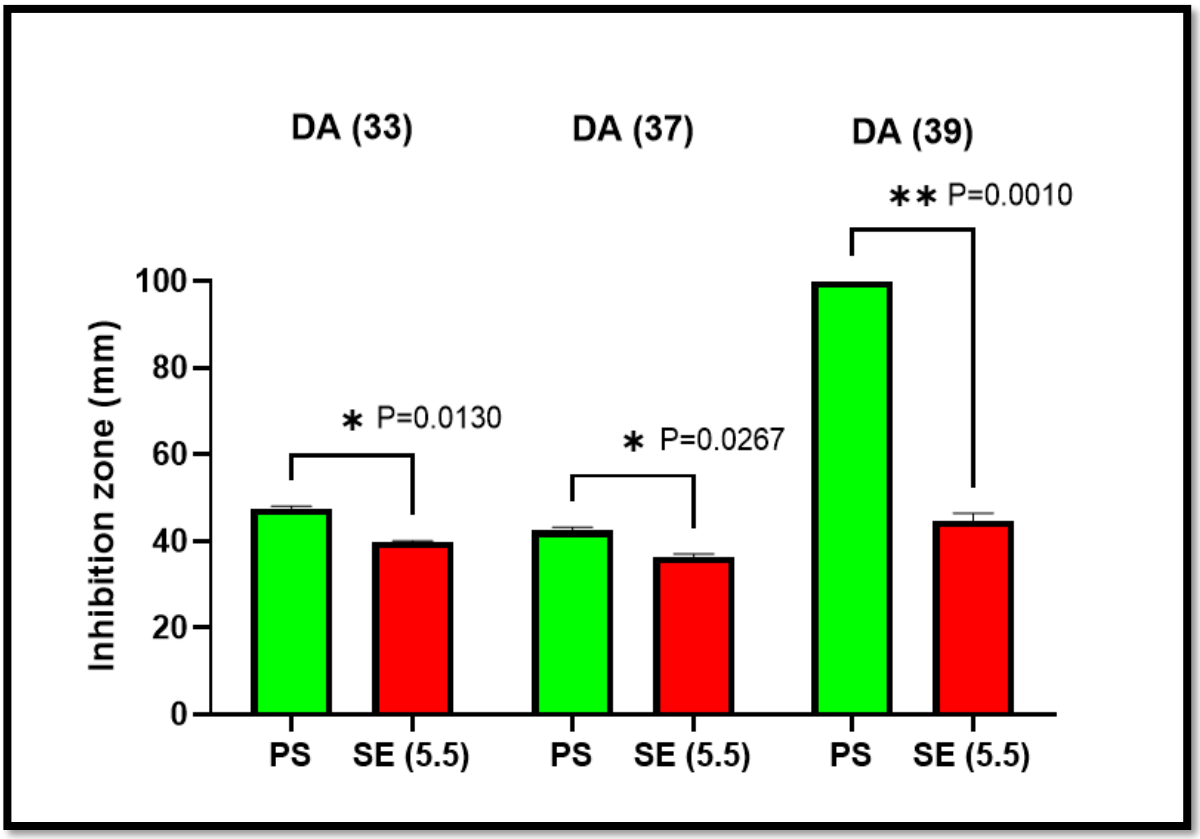

Figure. 31 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE5.5) on Clindamycin at three incubation temperatures- 33 °C, 37 °C, and 39 °C. This figure shows significant difference in antibiotic susceptibility at all temperatures (P=0.0130, DF=2, SD=1.528; P=0.0267, DF=2, SD=1.732; P=0.0010, DF=2, SD=3.055).

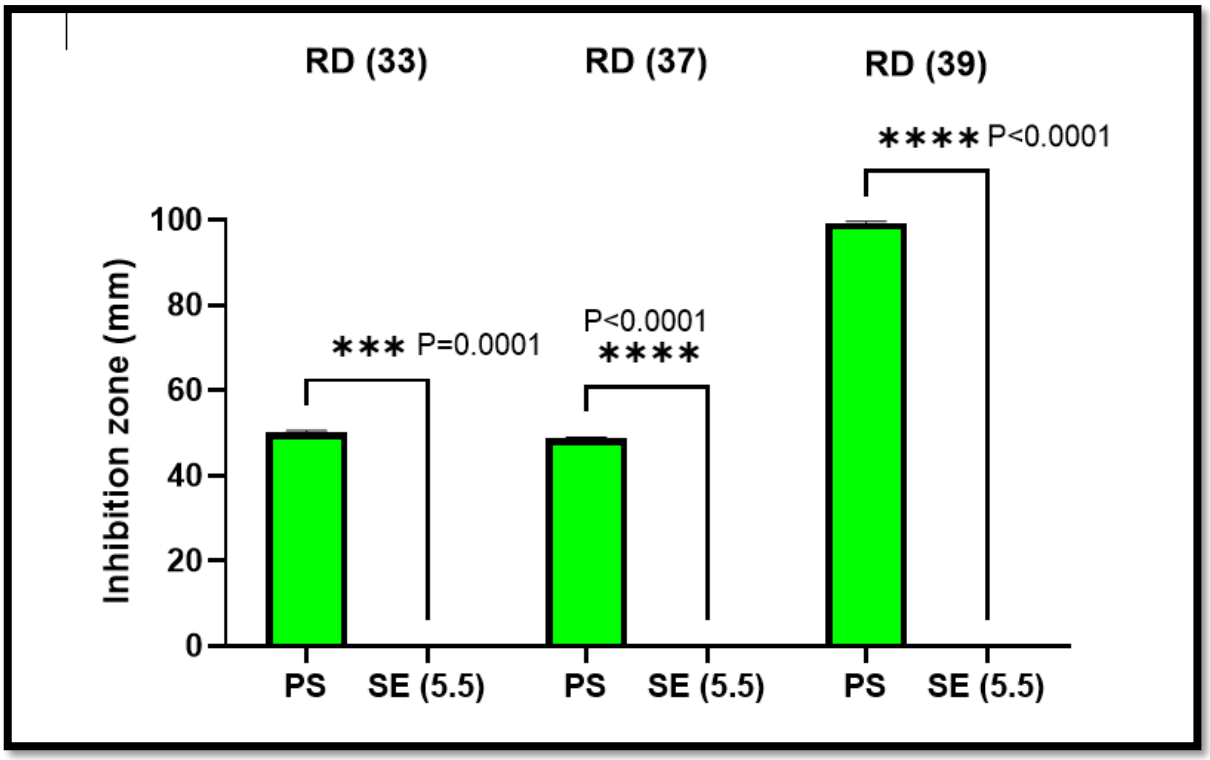

Figure. 32 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE5.5) on Rifampicin at three incubation temperatures- 33 °C, 37 °C, and 39 °C. This figure shows significant difference in antibiotic susceptibility at all temperatures (P=0.0001, DF=2, SD=1.000; P<0.0001, DF=2, SD=0.5774; P<0.0001, DF=2, SD=0.5774).

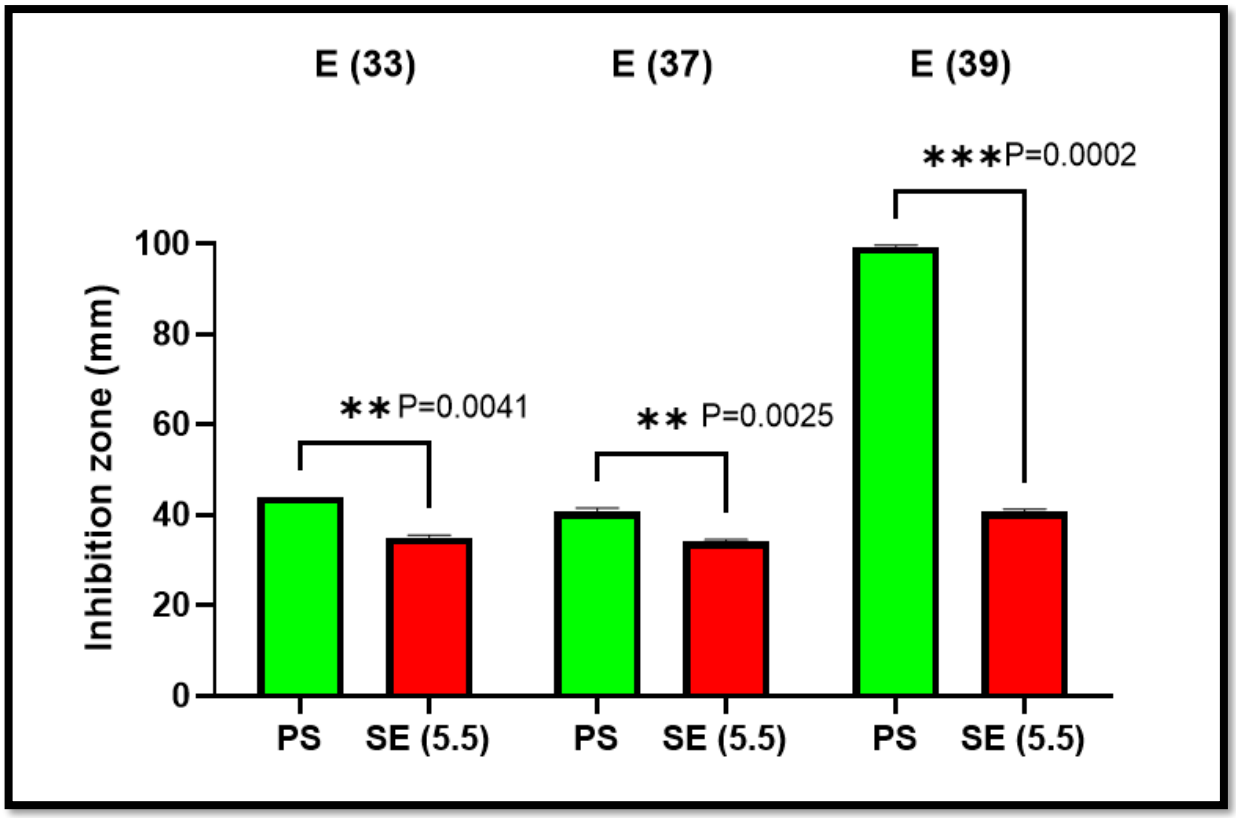

Figure. 33 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE5.5) on Erythromycin at three incubation temperatures- 33 °C, 37 °C, and 39 °C. This figure shows significant difference in antibiotic susceptibility at all temperatures (P=0.0041, DF=2, SD=1.000; P=0.0025, DF=2, SD=0.5774; P=0.0002, DF=2, SD=1.528).

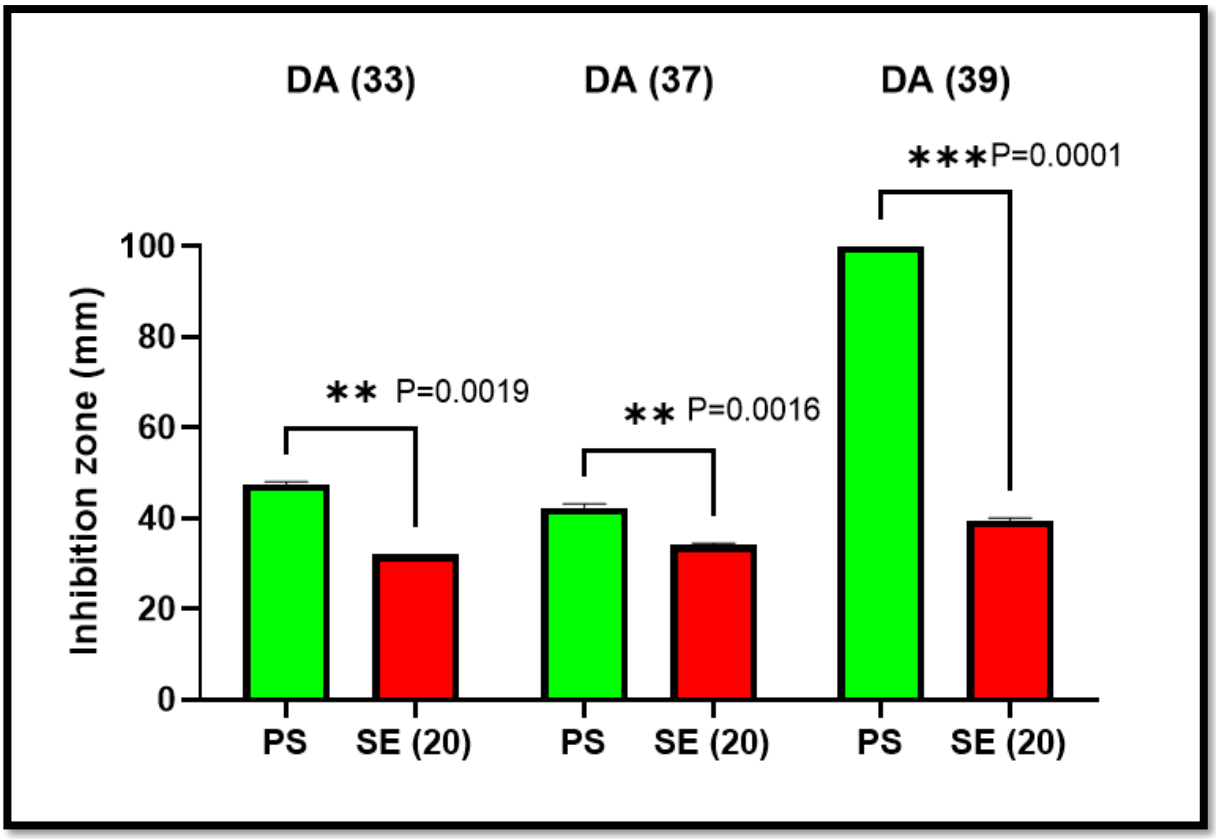

Figure. 34 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE20) on Clindamycin at three incubation temperatures- 33 °C, 37 °C, and 39 °C. This figure shows significant difference in antibiotic susceptibility at all temperatures (P=0.0019, DF=2, SD=1.155; P=0.0016, DF=2, SD=0.5774; P=0.0001, DF=2, SD=1.155).

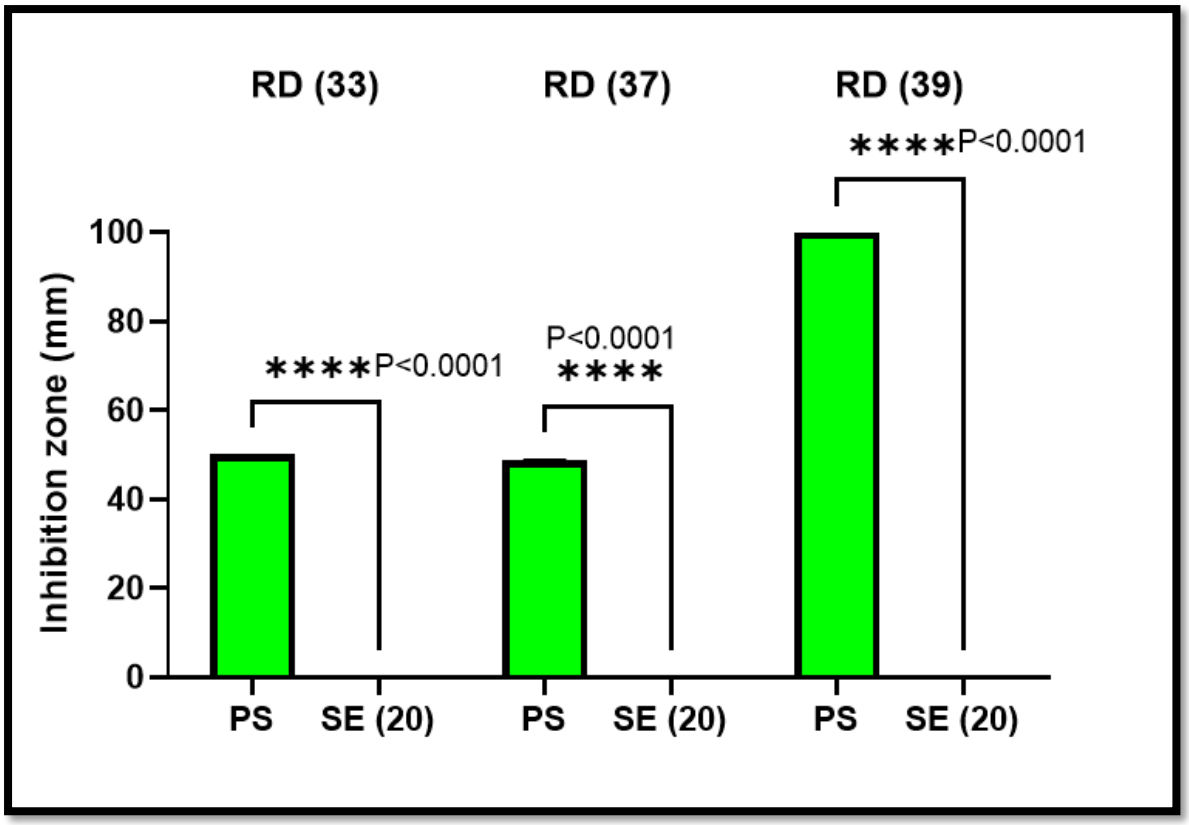

Figure. 35 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE20) on Rifampicin at three incubation temperatures- 33 °C, 37 °C, and 39 °C. This figure shows significant difference in antibiotic susceptibility at all temperatures (P<0.0001, DF=2, SD=0.005774; P<0.0001, DF=2, SD=0.5774; P<0.0001, DF=2, SD=0.005774).

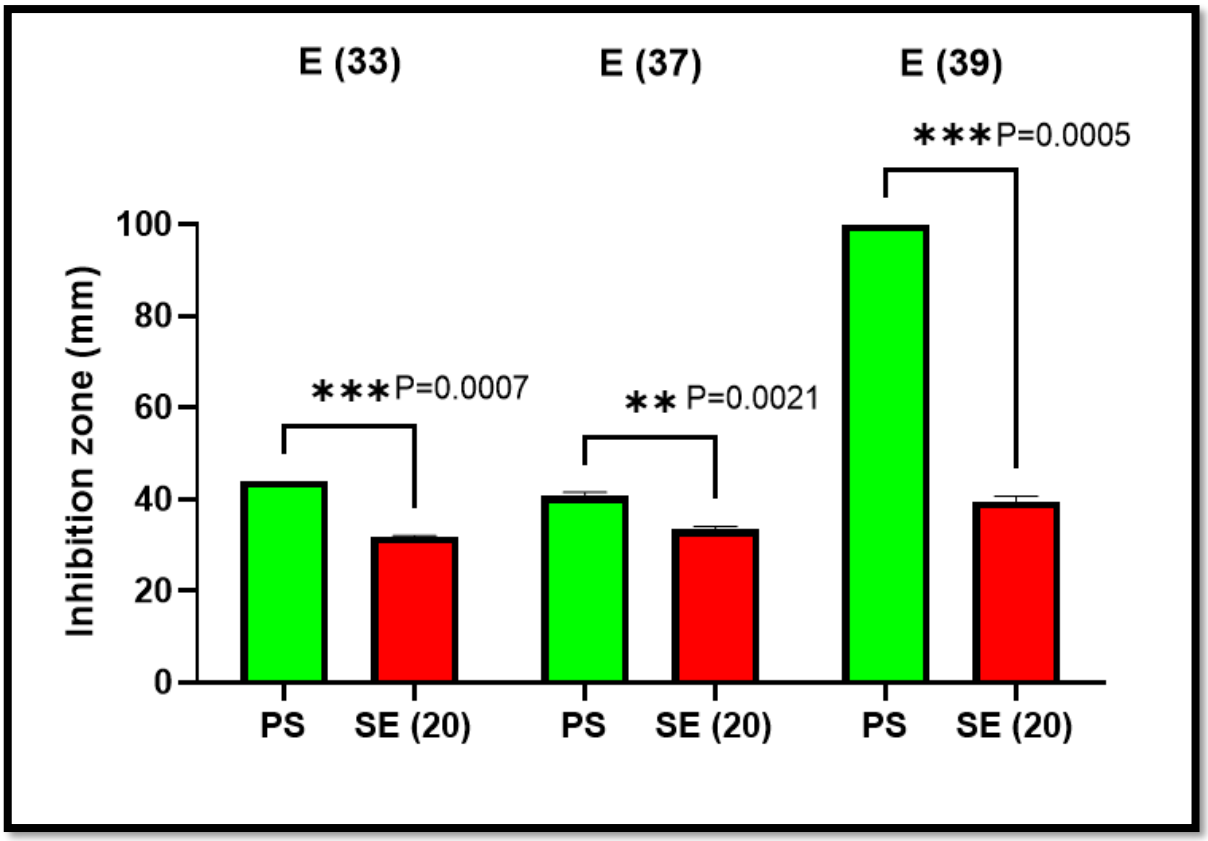

Figure. 36 Column bar graph showing the adaptation of *S. epidermidis* parent strain (PS) and adapted strain (SE20) on Erythromycin at three incubation temperatures- 33 °C, 37 °C, and 39 °C. This figure shows significant difference in antibiotic susceptibility at all temperatures (P=0.0007, DF=2, SD=0.5774; P=0.0021, DF=2, SD=0.5774; P=0.0005, DF=2, SD=2.309).

4. Discussion

Wound management has emerged as a significant concern in the last decades (Packer et al., 2024). More specifically, acute wounds are quite often implication of poor diabetes management-19% to 34% of the diabetic patients worldwide develop acute wounds in their lifetime (Meloni et al., 2020). Additionally, the most commonly prescribed wound treatment are wound-related antibiotics, as 53% to 71% of patients receive antibiotic at least once during their treatment (Khan et al., 2014; Price, 2020). However, multi-drug antibiotic resistance has emerged a lot in the recent years, resulting in treatment difficulties and the need of alternative therapies (Teng et al., 2020). Research has shown that glucose is able to affect bacterial burden and treatment efficacy (Fathollahipour et al., 2020; Mirhoseini et al., 2021; Müller et al.,

2013). More specifically, it is shown that Rifampicin and Erythromycin could be combined with honey, resulting in a significant increase in their efficacy (Fathollahipour et al., 2020; Müller et al., 2013). From the other hand, there is a research that suggest increased efficasy of Clindamycin combined with topical insulin (Mirhoseini et al., 2021). Therefore, there is a relationship between bacteria susceptibility and glucose, which might be the cause of unmanagable acute wounds in diabetic patients. Up to date there is no known research that has examined specifically the relationship between antibiotic susceptibility and glucose concentrations. Further, wound healing happens at lower pH level (Pivian Sim et al., 2022). Research has shown that there is a decrease in antibiotic's activity at lower pH, however its toxicity is being increased at basic millieu (Dissemond et al., 2005). Therefore, there is an important relationship between antibiotic susceptibility/effectiveness and pH. Additionally, temperature has a major effect on wound healing too. It is proven that 33°C to 35°C indicate wound recovery, however there is not enough evidence to suggest that temperature could be used as an ultimate indicator of wound healing process (Dini et al., 2015). Recent study shows that management of the pH, temperature and bacterial burden improves overall wound recovery (Derwin et al., 2023). The current study has showed that there is a significant relationship between skin microbiota's antibiotic susceptibility and these three factors-glucose, pH and temperature. Specifically, the chosen isolates of *S. epidermidis* and *S. aureus* have shown: i) lower susceptibility to antibiotics at higher glucose concentration; ii) lower susceptibility to antibiotics at lower pH (acidic millieu); iii) lower susceptibility to antibiotics at lower temperature.

As mentioned above, antibiotic resistance has become a significant global health crisis recently, rendering many infections increasingly difficult to treat (Talebi Bezmin Abadi et al., 2019). Furthermore, skin bacteria are being particularly impacted, as they can develop resistance leading to acute infections (Meneghetti et al., 2018). For example, *S. epidermidis*, as many *Staphylococci* species, have the ability to form biofilms (Patel et al., 2007). Biofilm formation significantly enhances the bacterial survival in the presence of antibiotics, making them more pathogenic (Cao et al., 2018). The first clinical isolate *S. epidermidis* (S5T36A TMS) tested for the current research showed resistance to 20 antibiotics (>3 antimicrobial classes). Therefore, this strain was considered as multi-drug resistant (Magiorakos et al., 2012). Bacterial biofilms contain huge amounts of extracellular DNA, and apart from acting as

preventative barrier, they allow the exchange of antibiotic resistance genes (de Araujo et al., 2006). This phenomenon contributes to the development of multi-drug resistance phenotypes, therefore mutant strains, which can cause untreatable infection (Subramanian et al., 2012). The development of new strategies for treating biofilm-associated infections, including biofilm inhibition and the screening of biofilm inhibitors, would provide important insights into their prevention and treatment (Rohde et al., 2005). Recent research suggests that the use of rifampicin-coated medical devices and antibiotic monotherapy in hospitals are the main promoters of antibiotic resistance development (Lee et al., 2018). Additionally, surgical implants are usually rifampicin, vancomycin, gentamicin coated, to prevent infections (Alt et al., 2014; Dhabuwala et al., 2011; Reinbold et al., 2017). However, the common use of these specific antimicrobials can easily lead to resistance development through adaptation (Bliziotis et al., 2005). As with oral and topical antibiotics, combination approach must be followed, specifically with antibiotics such as rifampicin and vancomycin (Stavrakis et al., 2014). Many studies have shown that a combination antibiotic therapy is ideal and very effective for patients with acute infections, caused by multi- and pan- drug resistant bacteria (Durante-Mangoni et al., 2013; Lim et al., 2011; Paul et al., 2014; Santimaleeworagun et al., 2011). Therefore, another example of how an unpathogenic commensal skin colonizer has the ability to become an important pathogenic bacteria (Sahal & Bilkay, 2014).

The initial hypothesis of the current study was that there is a negative correlation between glucose and antibiotic susceptibility. The results from the adaptation of both bacteria on Rifampicin proved this hypothesis, as there was strong significance between the initial isolate strains and the adapted ones. This means that adaptation on high glucose induce faster adaptation, leading to complete resistance in this specific case. Furthermore, the complete resistance occurred first at glucose concentration of 20mm, then at 5.5mm and then at 0mm, which shows again that glucose has a significant effect on antibiotic susceptibility. Although, there was no significant relationship statistically proven for the rest of the tested antibiotics, Clindamycin and Erythromycin, there was a trend in adaptation showing lower susceptibility on higher glucose concentrations for both bacteria. Moreover, *S. aureus* colonies increased their golden pigmentation, spesifically throughout the first passages. This golden pigmentation is related with the level of virulence-it impairs

neutrophil killing and promote virulence (Liu et al., 2005). Therefore, the observed increase and decrease in colour within the adaptation process is related with higher and lower pathogenicity/resistance to the antibiotics. In addition, the odour of the bacteria changed over time. Specifically, *S. epidermidis* colonies increased their odour towards the last passages, which indicates changes in their physiology. Literature suggests that odour is related to improved infection (Haalboom et al., 2019). For example, leg ulcers in diabetic patients are acute infected wounds with strong odour, which are difficult to be managed, and have huge negative impact on the patient's life (Oliveira et al., 2019). Therefore, the observed odour could indicate increased pathogenicity of the colonies, resulted by favourable selection of mutations through passaging. From the other hand, Rifampicin resistance has evolved a lot in the recent decades, having negative clinical impact (Van Rie et al., 2020). It is suggested that it has evolved mainly due to fitness-compensatory mutations in RNA polymerase (Kurepina et al., 2022). These mutations result in enhanced adaptation, more efficient transcription of the favourable genes (as RNA polymerase is crucial factor in gene expression), improved stress response etc (Kurepina et al., 2022). Rifampicin is suggested to be used in a combination with another antimicrobial to prevent resistance (Achermann et al., 2013; Skinner et al., 2017). Therefore, this accounts for the rapid development of resistance in both *S. aureus* and *S. epidermidis* isolates, occurring as early as the 4^{th} and 6^{th} passage, respectively. Additionally, recent study has examined the adaptation of *S. aureus* in an *in vitro* mimicking diabetic foot environment and found that increased glucose resulted in an increased expression of genes involved in biofilm formation (Pouget et al., 2021). As mentioned above, both bacteria are species that form biofilm to prevent antibiotic susceptibility (Le et al., 2019; McCourt et al., 2014). As a result, glucose induce biofilm formation, and the bacteria, through natural selection, develop fitness-cost related mutations. These mutations enable the bacteria to respond more effectively to future stresses encountered during passaging. On the other hand, glucose not only helps bacterial adaptation, but also antibiotic efficacy (Al Saeed, 2013; Peng et al., 2015; Zhang et al., 2020). Multiple studies have shown that glucose in different forms, combined with the initial antibiotic treatment, results in high increase in its toxicity (Alandejani et al., 2009; Pleeging et al., 2020). Specifically, Daptomycin in a combination with glucose increase its killing rate by up to 5-fold within one hour (Prax et al., 2016). In addition, research shows that the specific rifampicin-manuka honey combination is a superior to other antibiotic-honey combinations, as it

eradicates the bacterial biofilms (Liu et al., 2018). Collectively, if glucose benefits lower susceptibility, and glucose benefits antibiotic efficacy as well, then combination approach is needed to address the rising number of acute untreatable wound cases, specifically in diabetic patients.

As mentioned above, there are further factors that affects wound healing and treatment, such as skin/wound pH. The adapted to Rifampicin *S. epidermidis* strain's susceptibility was tested in three different pH levels- 5.5, 7.5, and 8.5. The same three antibiotics were tested- Rifampicin, Clindamycin, and Erythromycin. Comparisons between the parent strain and the three strains adapted to different glucose concentrations (0mm, 5.5mm, 20mm) were made. The initial hypothesis was a negative correlation between pH and antibiotic susceptibility. The results showed a negative correlation for the parent strain and the adapted at 20mm glucose strain for the antibiotics Clindamycin and Rifampicin. However, a positive correlation was observed for the parent strain and the adapted strains at 0mm and 5.5mm glucose for the antibiotics Erythromycin and Clindamycin, along with the adapted at 20mm glucose strain for Erythromycin as well. This indicates that pH could affect treatment differently. The hypothesis can be rejected, as most of the results are opposite to it, however there are some results that prove that the hypothesis is right. Study has tested the strips of rat superficial fascia and their responses *in vitro* at pH 5.5, 6.1, 7.3, and 8.1 (Pipelzadeh & Naylor, 1998). The obtained results showed enhanced myofibroblast contractility (vital for cutaneous wound healing) at acidic millieu (Pipelzadeh & Naylor, 1998). Another study has shown that the antimicrobial activity of silver dressing for MRSA is significantly enhanced at pH 5.5 (Percival et al., 2011). Furthermore, increased pH is associated with wound infection in second degree burns (Ono et al., 2015). These indicate that wound healing and increased antibiotic efficacy occurs at acidic millieu. However, most of the results of the current study showed lower susceptibility at lower pH. Research has examined the antibiotic susceptibillity of *Salmonella* species to *β*-lactams at different pH values and found increased resistance at acidic millieu (Laub et al., 1989). The authors suggest that this resistance has arisen due to changes in the permeability of the bacterial outer membrane to antibiotics (Laub et al., 1989). Recent research has shown that acidic conditions induce biofilm formation in *Pseudomonas aeruginosa*, therefore antibiotic resistance (Lin et al., 2021). Low pH has been found to promote the increased production of ampicillin-

resistant persisters in *E. coli* (Hong et al., 2012). In addition, low pH has been found to induce gene encoding multidrug resistance to Moxifloxacin in *S. aureus* (Truong-Bolduc et al., 2011). Therefore, this suggests a major concern in wound healing practices. Furthermore, adapting an already adapted strains to specific antibiotic, to different antibiotics, could lead to cross-resistance. As all strains, at all glucose concentration were fully adapted to the Rifampicin, this pre-exposure could have increased the further antibiotic's resistance. However, no cross-resistance was observed at these conditions. This phenomenon must be considered in clinical settings due to its significant impact on treatment efficacy (Obolski et al., 2016). Recent research has shown that animal-use antibiotics induce cross-resistance in bacterial pathogens to human antibiotics (Singh & Bhunia, 2019). More specifically, they found that pre-exposure to Tilmicosin and Florfenicol, increase cross-resistance from 1.25 to 40-fold, against Ampicillin, Tetracycline and Nalidixic acid (Singh & Bhunia, 2019). Collectively, if there is a negative correlation between glucose and antibiotic susceptibility (fully confirmed from the adaptation to Rifampicin), and there is a positive correlation between pH and antibiotic susceptibility (fully confirmed from the adaptation to Erythromycin), then is there any correlation between high glucose adapted strain's antibiotic susceptibility and pH? The results from the adaptation to Erythromycin showed that the adapted strain at 20mm glucose concentration was less susceptible at lower pH, however the results from the adaptation to Clindamycin at high glucose showed higher susceptibility at lower pH. In comparison with the parent strain there was a significant decrease in susceptibility (P=0.013) and significant increase in susceptibility (P=0.002) at lower pH, respectively. Therefore, a positive and negative correlation could be confirmed, dependent on the treatment used. A higher susceptibility at lower pH (negative correlation) indicates succesfull treatment, as wound healing and increased antibiotic efficacy commonly occur at acidic pH, as mentioned above. However, a positive correlation could indicate a major concern for diabetic patients in clinical practice. *Staphylococcus* species are gram-positive bacteria and the most routinely used treatment for diabetic foot ulcers is Erythromycin (16%) (Al Ayed et al., 2018; Edmonds & Foster, 2004; Murugan et al., 2008). Additionally, Erythromycin is a macrolide antibiotic, and this group of antibiotics is known to lose 90% of their activity for each drop of 1 U of pH (Smith et al., 2000). A recent study has examined the degradation rate of Erythromycin at different pH levels and faster degradation was observed at pH 5 (Chu et al., 2019). Therefore, macrolides,

such as Erythromycin are not effective at low pH, and are unappropriate for treatment, specifically in diabetic acute wounds.

Another important factor affecting wound healing and treatment effectiveness is wound temperature (Brooks et al., 2021). Again, the adapted to Rifampicin *S. epidermidis* strain's susceptibility was tested at three different temperatures- 33 °C, 37 °C, and 39 °C. The same three antibiotics were tested- Rifampicin, Clindamycin, and Erythromycin. Comparisons between the parent strain and the three strains adapted to different glucose concentrations (0mm, 5.5mm, 20mm) were made. The initial hypothesis was a negative correlation between temperature and antibiotic susceptibility. However, the results showed overall lower susceptibility at lower temperature for all strains. Therefore, the initial hypothesis was rejected. Additionally, for the control strain (adapted to 0 mm glucose), little to no growth was observed, so sufficient results could not be obtained. Thus, significant difference between the parent strain and the adapted strains (at 5.5mm and 20mm glucose) was observed at all temperatures and antibiotics. A recent study has found that antibiotics are able to shift the temperature response curve of bacterial growth (Cruz-Loya et al., 2021). The scientists have measured the growth of an *Escherichia coli* strain under 12 different antibiotics across 7 temperatures within the range of 22°C to 46°C (Cruz-Loya et al., 2021). The results showed that for example the optimal growth curve under Erythromycin, compared to the optimal growth curve under no drug, had a right shift (optimal growth at higher temperature), but under Clindamycin- the optimal growth temperature was shifted to the left (Cruz-Loya et al., 2021). Further, this study suggests that both temperature and drug stress create a shared-damage, when an antibiotic damage the same cellular components as hot or cold temperature does (Cruz-Loya et al., 2021). As known, wound healing takes place at low temperatures, along with higher antibiotic efficacy (G Nakagami et al., 2010; Nakaminami et al., 2019). However, the results of the current study showed that all strains were less susceptible to antibiotics at lower temperature conditions. This could indicate a major issue in wound healing and treatment effectiveness. Very recent research has shown that *E. coli* exhibits multidrug resistance to Gatifloxacin at 27 °C, with a 256-fold increase (Zhao et al., 2024). Additionally, an increase in biofilm formation ability and intracellular mutation rates were observed (Zhao et al., 2024). More antibiotics, such as Rifampicin and Erythromycin were tested, and unique mutations were observed at

different temperatures (Zhao et al., 2024). It is important to be mentioned that mutations to certain antibiotics occurred in drug-free environment, due inheritance and/or selective pressure (Zhao et al., 2024). *E. coli* is able to develop Rifampicin resistance under antibiotic-free environment at high temperatures (thermal stress), which indicates that extreme temperatures (low and high) could lead to the development of unique resistance genes (Rodríguez-Verdugo et al., 2013). An association between antibiotic resistance and climate change has been suggested in the past couple of years, however it remains inconclusive and further research is needed (Kaba et al., 2020; Li et al., 2023). Therefore, if there is a negative correlation between glucose and antibiotic susceptibility, and a positive correlation between temperature and antibiotic susceptibility, then is there any relationship between high glucose adapted strain's antibiotic susceptibility and temperature? The results of the current study show that the adapted to 20mm glucose *S. epidermidis* strain was least susceptible to Clindamycin and Erythromycin at 33 °C, compared to 37 °C and 39 °C. Furthermore, there was a significant difference in susceptibility between the parent and adapted strain for both antibiotics, at all temperatures. Therefore, there is a positive correlation between high glucose adapted strain's antibiotic susceptibility and temperature. This finding indicates major clinical concern, specifically for diabetic patients. A study has tested the biofouling of several biofilm forming bacteria, such as *Staphyloccoci* species and *E. coli*, when exposed to the most common SS materials used in the medical industries, under different temperature and glucose conditions (Bezek et al., 2019). The authors observed a significant increase in biofouling by an *S. aureus* strain when grown in 5% glucose after 48 hours of incubation, with the same effect observed after 24 hours of incubation at 22°C (Bezek et al., 2019). Furthermore, there are recent studies that suggest pH and temperature management increase wound healing rates (Derwin et al., 2023). However, there are no studies, to our knowledge, that specifically suggests management of pH, temperature and glucose concentrations in order to increase acute wounds treatment's effectiveness. Novel treatments, such as pH and temperature responsive hydrogels are recently suggested to effectively cure acute wound infections (Haidari et al., 2022).

As all studies, the current research has its own limitations. One of the main limitations is that the hypotheses were tested in an environment not relevant to the human host. Laboratory experiments are run under fixed and managable conditions, an

environment quite different of what actually happens in the human body. These experiments are not able to represent and test the interactions between the host and the skin microbiome, along with all different biological processes, which take place in homeostasis etc. Relevant studies have used animal models, such as BALB/c mice or pig, however these species have skin microbiome similar to human, but not closely related (Lundberg & Frimodt-Møller, 2013; Wareham-Mathiassen et al., 2023). A recent study has examined the skin microbiome of 38 species across 10 mammalian orders and found that they have much more diverse microbiomes than humans, due to habitat, environmental conditions, and evolution (Ross et al., 2018). More specifically, the authors found that human samples were dominated by bacteria, such as *S. epidermidis*, *Corynebacterium*, and *P. acnes*, thus the primate and other mammalian species microbiome had higher levels of traditional soil-associated OTUs (Council et al., 2016; Ross et al., 2018). Therefore, animal models are not appropriate for testing skin microbiome-host interactions, and closely related alternative is needed. Additionally, the disk diffusion method used to test the bacterial susceptibility to antibiotics is commonly used, but yet it has some limitations. Proffesional training of perfoming the test and reading the results is required, as human errors could lead to false results, specifically when applied in clinical settings (Edelmann et al., 2007). Furthermore, despite good categorical classifications, it may exhibit slightly greater variability in reproducibility compared to the Etest or agar dilution test (Liu et al., 2016). To address the limitations of disk diffusion testing, the implementation of standardized, automated methods for direct susceptibility testing of clinical isolates that eliminate the need for retesting from subcultures, is often being suggested (Edelmann et al., 2007). However, in the current study, the same batch of antimicrobial disks was consistently used throughout the experiments. Further, although different batches of agar plates were used, there is no indication that this variation has significantly impacted the results. Additionally, since all tests were performed and read by a single researcher, no increased variability is expected, unlike the potential variation that could occur if multiple researchers were involved in interpreting the results. Consequently, follow-on studies need to be conducted and further assays/methods need to be used to test our hypotheses. As mentioned earlier, animal models could not mimic adequately the human host-skin microbiome interactions. Organs-on-chips (OoCs) have developed drastically in the last decade (Grassart et al., 2019). These novel devices are able to provide insights into human organ function and disease pathophysiology and are able

to accurately predict the safety and efficacy of different drugs and treatments in humans (Grassart et al., 2019). Therefore, they have the potential to become a replacement of the in vivo animal testing in the longer term. Recent study has shown that reconstructed human skin (RHS) and a liver model cultured in TissUse' HUMIMIC Chip2, as a combination, allowed increased sensitivity assessment of the skin and hepatic effects caused by chemicals able to pass through the skin (toxicity) (Tavares et al., 2020). Additionally, there are already studies that have used this method, along with 3D tissue-engineered models, to test effectively the antibiotic susceptibility of different bacteria (Kurow et al., 2023; Lu et al., 2013; Perebikovsky et al., 2021). Additionally, biofilm formation has been concluded as one of the major factors causing bacterial resistance to antimicrobials. However, in the current study the biofilm fromation and composition were not tested. High-throughput sequencing should be used in future studies to give more insights into the interactions between antibiotic susceptibility, pH, temperature and glucose. Recent research shows that the adaptation of *E. coli* to Triclosan has largely impacted its membrane properties, efflux and antibiotic resistance (Sonbol et al., 2019). Specifically, the results showed increased resistance, along with lower outer and inner membrane permeability (Sonbol et al., 2019). In addition, another research showed that *S. lugdunensis* adjusted its plasma membrane fatty acid composition, growth rates, and morphology in response to changing environment (pH, temperature, osmotic pressure), therefore achieving optimal adaptations for survival (Crompton et al., 2014). Whole-genome sequencing is a method that could detect unique mutations in the bacterial biofilms, thus revealing important interactions between the environmental stresses, biofilm pathophysiology and potential resistance mutations (Fauzia et al., 2023; Qi et al., 2016). Therefore, organ-on-chip models and high-throughput sequencing could be implied to further examine the results and hypotheses of the current study.

Consequently, the treatment of acute wounds is affected by multiple factors, such as glucose concentrations, pH, temperature, and bacterial burden, thus a combination treatment approach is required. However, preference of combination approaches over traditional treatment, will have several impacts. Initially, it will have quite positive clinical impact, as this approach will result in enhanced clinical outcomes, reduced resistance (multiple therapeutic agents) and personalized medicine (Vestergaard et al., 2016). Nevertheless, it will lead to increased complexity, as it will require more

specialized knowledge and the coordination of multiple treatments. Increased treatment cost might negatively impact the economy, however although initial costs might be higher, the long-term benefits of successful treatments, potentially lead to cost savings (cost-effectiveness) (Akazawa & Fukuoka, 2013; Wu et al., 2018). Social and ethical concerns might be raised, if increased costs affect the access to these treatments, potentially leading to inequalities in clinical care (Tran et al., 2016).

Conclusion

The current study emphasizes that optimizing treatment for acute wound infections requires considering multiple factors beyond just bacterial burden, such as wound pH, temperature, and glucose concentrations. Additionally, a detailed investigation of bacterial pathophysiology and virulence is necessary, along with an understanding of the interactions between antibiotic treatment, environmental stresses, and the bacteria itself (including biofilms and mutations). Rapid point-of-care (PoC) diagnostics for antibiotic susceptibility testing (AST) are essential in addressing the antimicrobial resistance epidemic. Consequently, follow-up studies should employ high-throughput sequencing, organ-on-chip devices, and 3D tissue-engineered models or microfluidic assays to explore various aspects of the issue. Finally, using closely related models and detailed assays can provide new insights into bacterial resistance in clinical settings, potentially leading to personalized medicine.

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References

Achermann, Y., Eigenmann, K., Ledergerber, B., Derksen, L., Rafeiner, P., Clauss, M., Nüesch, R., Zellweger, C., Vogt, M., & Zimmerli, W. (2013). Factors associated with rifampin resistance in staphylococcal periprosthetic joint infections (PJI): a matched case–control study. *Infection*, *41*, 431-437.

Akbas, M. Y., & Kokumer, T. (2015). The prevention and removal of biofilm formation of Staphylococcus aureus strains isolated from raw milk samples by citric acid treatments. *International Journal of Food Science & Technology*, *50*(7), 1666-1672.

Al-Hamamy, H. R., Sharquie, K. E., Noaimi, A. A., & Hussein, W. N. (2014). Topical erythromycin-zinc acetate complex lotion versus topical erythromycin gel in treatment of mild to moderate acne vulgaris. *Our Dermatology Online*, *5*(4), 347.

Al Ayed, M. Y., Ababneh, M., Alwin Robert, A., Alzaid, A., Ahmed, R. A., Salman, A., Musallam, M. A., & Al Dawish, M. A. (2018). Common pathogens and antibiotic sensitivity profiles of infected diabetic foot ulcers in Saudi Arabia. *The International Journal of Lower Extremity Wounds*, *17*(3), 161-168.

Al Saeed, M. (2013). Therapeutic efficacy of conventional treatment combined with Manuka honey in the treatment of patients with diabetic foot ulcers: a randomized controlled study. *The Egyptian Journal of Hospital Medicine*, *53*(1), 1064-1071.

Alandejani, T., Marsan, J., Ferris, W., Slinger, R., & Chan, F. (2009). Effectiveness of honey on Staphylococcus aureus and Pseudomonas aeruginosa biofilms. *Otolaryngology—Head and Neck Surgery*, *141*(1), 114-118.

Alt, V., Kirchhof, K., Seim, F., Hrubesch, I., Lips, K. S., Mannel, H., Domann, E., & Schnettler, R. (2014). Rifampicin–fosfomycin coating for cementless endoprostheses: Antimicrobial effects against methicillin-sensitive Staphylococcus aureus (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA). *Acta biomaterialia*, *10*(10), 4518-4524.

Andersson, D. I., & Hughes, D. (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature reviews microbiology*, *8*(4), 260-271.

Andreatos, N., Shehadeh, F., Pliakos, E. E., & Mylonakis, E. (2018). The impact of antibiotic prescription rates on the incidence of MRSA bloodstream infections: a county-level, US-wide analysis. *International journal of antimicrobial agents*, *52*(2), 195-200.

AW, N. H. H., Intan, N., Syafinaz, A., Zalinah, A., Akmar, L., & Devnani, A. S. (2015). Clinical presentation and microorganisms sensitivity profile for diabetic foot ulcers: a pilot study. *The Medical Journal of Malaysia*, *70*(3), 182-187.

Baxter, M., Bethune, C., Powell, R., & Morgan, M. (2020). Point prevalence of penicillin allergy in hospital inpatients. *Journal of Hospital Infection*, *106*(1), 65-70.

Beaber, J. W., Hochhut, B., & Waldor, M. K. (2004). SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature*, *427*(6969), 72-74.

Bedhomme, S., Amorós-Moya, D., Valero, L. M., Bonifaci, N., Pujana, M.-À., & Bravo, I. G. (2019). Evolutionary changes after translational challenges imposed by horizontal gene transfer. *Genome Biology and Evolution*, *11*(3), 814-831.

Bezek, K., Nipič, D., Torkar, K. G., Oder, M., Dražić, G., Abram, A., Žibert, J., Raspor, P., & Bohinc, K. (2019). Biofouling of stainless steel surfaces by four common pathogens: The effects of glucose concentration, temperature and surface roughness. *Biofouling*, *35*(3), 273-283.

Bliziotis, I. A., Samonis, G., Vardakas, K. Z., Chrysanthopoulou, S., & Falagas, M. E. (2005). Effect of aminoglycoside and β-lactam combination therapy versus β-lactam monotherapy on the emergence of antimicrobial resistance: a meta-analysis of randomized, controlled trials. *Clinical Infectious Diseases*, *41*(2), 149-158.

Brooks, E., Burns, M., Ma, R., Scholten, H. J., & Becker, S. (2021). Remote diabetic foot temperature monitoring for early detection of diabetic foot ulcers: a cost-effectiveness analysis. *ClinicoEconomics and Outcomes Research*, 873-881.

Brown, J. M., & Poston, S. M. (1983). Resistance of propionibacteria to antibiotics used in the treatment of acne. *Journal of Medical Microbiology*, *16*(3), 271-280.

Bunch, R. L., & Mcguire, J. M. (1953). Erythromycin, its salts, and method of preparation. In: Google Patents.

Burgess, J. L., Wyant, W. A., Abdo Abujamra, B., Kirsner, R. S., & Jozic, I. (2021). Diabetic wound-healing science. *Medicina*, *57*(10), 1072.

Candler, T., Mahmoud, O., Lynn, R., Majbar, A., Barrett, T., & Shield, J. (2018). Continuing rise of type 2 diabetes incidence in children and young people in the UK. *Diabetic Medicine*, *35*(6), 737-744.

Cao, Y., Su, B., Chinnaraj, S., Jana, S., Bowen, L., Charlton, S., Duan, P., Jakubovics, N. S., & Chen, J. (2018). Nanostructured titanium surfaces exhibit recalcitrance towards Staphylococcus epidermidis biofilm formation. *Scientific reports*, *8*(1), 1071.

Cau, L., Williams, M. R., Butcher, A. M., Nakatsuji, T., Kavanaugh, J. S., Cheng, J. Y., Shafiq, F., Higbee, K., Hata, T. R., & Horswill, A. R. (2021). Staphylococcus epidermidis protease EcpA can be a deleterious component of the skin microbiome in atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *147*(3), 955-966. e916.

Chen, W., Xie, T.-T., & Zeng, H. (2019). Formation, antibiotic resistance, and control strategies of Staphylococcus epidermidis biofilm. In *Bacterial Biofilms*. IntechOpen.

Chu, L., Zhuan, R., Chen, D., Wang, J., & Shen, Y. (2019). Degradation of macrolide antibiotic erythromycin and reduction of antimicrobial activity using persulfate activated by

gamma radiation in different water matrices. *Chemical Engineering Journal*, *361*, 156-166.

Conlan, S., Mijares, L. A., gov, N. C. S. P. m. m. n., Becker, J., Blakesley, R. W., Bouffard, G. G., Brooks, S., Coleman, H., Gupta, J., & Gurson, N. (2012). Staphylococcus epidermidis pan-genome sequence analysis reveals diversity of skin commensal and hospital infection-associated isolates. *Genome biology*, *13*, 1-13.

Council, S. E., Savage, A. M., Urban, J. M., Ehlers, M. E., Skene, J. P., Platt, M. L., Dunn, R. R., & Horvath, J. E. (2016). Diversity and evolution of the primate skin microbiome. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1822), 20152586.

Crompton, M. J., Dunstan, R. H., Macdonald, M. M., Gottfries, J., von Eiff, C., & Roberts, T. K. (2014). Small changes in environmental parameters lead to alterations in antibiotic resistance, cell morphology and membrane fatty acid composition in Staphylococcus lugdunensis. *PloS one*, *9*(4), e92296.

Cruz-Loya, M., Tekin, E., Kang, T. M., Cardona, N., Lozano-Huntelman, N., Rodriguez-Verdugo, A., Savage, V. M., & Yeh, P. J. (2021). Antibiotics shift the temperature response curve of Escherichia coli growth. *Msystems*, *6*(4), 10.1128/msystems. 00228-00221.

Czarnecka, K., Lisiecki, P., Szewczyk, E., Chufarova, N., Wójtowicz, P., Kręcisz, P., & Szymański, P. (2020). New acridine derivatives as promising agents against methicillin-resistant staphylococci–From tests to in silico analysis. *Computational Biology and Chemistry*, *88*, 107321.

Dang, C., Prasad, Y., Boulton, A., & Jude, E. (2003). Methicillin-resistant Staphylococcus aureus in the diabetic foot clinic: a worsening problem. *Diabetic Medicine*, *20*(2), 159-161.

de Araujo, G. L., Coelho, L. R., de Carvalho, C. B., Maciel, R. M., Coronado, A. Z., Rozenbaum, R., Ferreira-Carvalho, B. T., Sá Figueiredo, A. M., & Teixeira, L. A. (2006). Commensal isolates of methicillin-resistant Staphylococcus epidermidis are also well equipped to produce biofilm on polystyrene surfaces. *Journal of Antimicrobial Chemotherapy*, *57*(5), 855-864.

de Souza, R. F. B., de Souza, F. C. B., & Moraes, Â. M. (2016). Polysaccharide-based membranes loaded with erythromycin for application as wound dressings. *Journal of Applied Polymer Science*, *133*(22).

Derwin, R., Patton, D., Strapp, H., & Moore, Z. (2023). The effect of inflammation management on pH, temperature, and bacterial burden. *International Wound Journal*, *20*(4), 1118-1129.

Dhabuwala, C., Sheth, S., & Zamzow, B. (2011). Infection rates of rifampin/gentamicin-coated Titan Coloplast penile implants. Comparison with Inhibizone-impregnated AMS penile implants. *The Journal of Sexual Medicine*, *8*(1), 315-320.

Di Domizio, J., Belkhodja, C., Chenuet, P., Fries, A., Murray, T., Mondéjar, P. M., Demaria, O., Conrad, C., Homey, B., Werner, S., Speiser, D. E., Ryffel, B., & Gilliet, M. (2020). The commensal skin microbiota triggers type I IFN-dependent innate repair responses in injured skin. *Nat Immunol*, *21*(9), 1034-1045. https://doi.org/10.1038/s41590-020-0721-6

Diehr, S., Hamp, A., & Jamieson, B. (2007). Do topical antibiotics improve wound healing?

Dini, V., Salvo, P., Janowska, A., Di Francesco, F., Barbini, A., & Romanelli, M. (2015). Correlation between wound temperature obtained with an infrared camera and clinical wound bed score in venous leg ulcers. *Wounds: a compendium of clinical research and practice*, *27*(10), 274-278.

Dissemond, J., Koppermann, M., Esser, S., Schultewolter, T., Goos, M., & Wagner, S. (2002). Therapie eines Methicillin-resistenten Staphylococcus aureus (MRSA) im Rahmen der Behandlung eines chronischen Ulkus mittels Biochirurgie. *Der Hautarzt*, *53*(9), 608-612.

Dissemond, J., Körber, A., Lehnen, M., & Grabbe, S. (2005). Methicillin-resistenter Staphylococcus aureus (MRSA) in chronischen Wunden: Therapeutische Optionen und Perspektiven: Methicillin-resistant Staphylococcus aureus (MRSA) in chronic wounds: Therapeutic options and perspectives. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, *3*(4), 256-262.

Duong, M., Markwell, S., Peter, J., & Barenkamp, S. (2010). Randomized, controlled trial of antibiotics in the management of community-acquired skin abscesses in the pediatric patient. *Annals of emergency medicine*, *55*(5), 401-407.

Durante-Mangoni, E., Signoriello, G., Andini, R., Mattei, A., De Cristoforo, M., Murino, P., Bassetti, M., Malacarne, P., Petrosillo, N., & Galdieri, N. (2013). Colistin and rifampicin compared with colistin alone for the treatment of serious infections due to extensively drug-resistant Acinetobacter baumannii: a multicenter, randomized clinical trial. *Clinical Infectious Diseases*, *57*(3), 349-358.

Edelmann, A., Pietzcker, T., & Wellinghausen, N. (2007). Comparison of direct disk diffusion and standard microtitre broth dilution susceptibility testing of blood culture isolates. *Journal of Medical Microbiology*, *56*(2), 202-207.

Edmonds, M., & Foster, A. (2004). The use of antibiotics in the diabetic foot. *The American journal of surgery*, *187*(5), S25-S28.

Ellis, S., Lin, E. J., & Tartar, D. (2018). Immunology of wound healing. *Current dermatology reports*, *7*, 350-358.

Faghihi, G., Isfahani, A. K., Hosseini, S. M., & Radan, M. R. (2012). Efficacy of intense pulsed light combined with topical erythromycin solution 2% versus topical erythromycin solution 2% alone in the treatment of persistent facial erythematous acne macules. *Advanced Biomedical Research*, *1*(1), 70.

Fathollahipour, S., Koosha, M., Tavakoli, J., Maziarfar, S., & Mehrabadi, J. F. (2020). Erythromycin releasing PVA/sucrose and PVA/honey hydrogels as wound dressings with antibacterial activity and enhanced bio-adhesion. *Iranian Journal of Pharmaceutical Research: IJPR*, *19*(1), 448.

Fauzia, K. A., Aftab, H., Miftahussurur, M., Waskito, L. A., Tuan, V. P., Alfaray, R. I., Matsumoto, T., Yurugi, M., Subsomwong, P., & Kabamba, E. T. (2023). Genetic determinants of Biofilm formation of Helicobacter pylori using whole-genome sequencing. *BMC microbiology*, *23*(1), 159.

Fox, C. S., Coady, S., Sorlie, P. D., Levy, D., Meigs, J. B., D'Agostino, R. B., Wilson, P. W., & Savage, P. J. (2004). Trends in cardiovascular complications of diabetes. *Jama*, *292*(20), 2495-2499.

Franks, P., Rolandsson, O., Debenham, S., Fawcett, K., Payne, F., Dina, C., Froguel, P., Mohlke, K., Willer, C., & Olsson, T. (2008). Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. *Diabetologia*, *51*, 458-463.

Germain, E., Castro-Roa, D., Zenkin, N., & Gerdes, K. (2013). Molecular mechanism of bacterial persistence by HipA. *Molecular cell*, *52*(2), 248-254.

Giacco, F., & Brownlee, M. (2011). Mechanisms of hyperglycemic damage in diabetes. *Atlas of Diabetes: Fourth Edition*, 217-231.

Gifford, D. R., Krašovec, R., Aston, E., Belavkin, R. V., Channon, A., & Knight, C. G. (2018). Environmental pleiotropy and demographic history direct adaptation under antibiotic selection. *Heredity*, *121*(5), 438-448.

Gjini, E., & Brito, P. H. (2016). Integrating antimicrobial therapy with host immunity to fight drug-resistant infections: classical vs. adaptive treatment. *PLoS computational biology*, *12*(4), e1004857.

Grassart, A., Malardé, V., Gobaa, S., Sartori-Rupp, A., Kerns, J., Karalis, K., Marteyn, B., Sansonetti, P., & Sauvonnet, N. (2019). Bioengineered human organ-on-chip reveals intestinal microenvironment and mechanical forces impacting Shigella infection. *Cell host & microbe*, *26*(3), 435-444. e434.

Haalboom, M., Gerritsen, J., & van der Palen, J. (2019). Differentiation between infected and non-infected wounds using an electronic nose. *Clinical Microbiology and Infection*, *25*(10), 1288. e1281-1288. e1286.

Haidari, H., Vasilev, K., Cowin, A. J., & Kopecki, Z. (2022). Bacteria-activated dual pH-and temperature-responsive hydrogel for targeted elimination of infection and improved wound healing. *ACS Applied Materials & Interfaces*, *14*(46), 51744-51762.

Halbert, A. R., Stacey, M. C., Rohr, J. B., & Jopp-Mckay, A. (1992). The effect of bacterial colonization on venous ulcer healing. *Australasian journal of dermatology*, *33*(2), 75-80.

Händel, N., Schuurmans, J. M., Brul, S., & ter Kuile, B. H. (2013). Compensation of the metabolic costs of antibiotic resistance by physiological adaptation in Escherichia coli. *Antimicrobial agents and chemotherapy*, *57*(8), 3752-3762.

Händel, N., Schuurmans, J. M., Feng, Y., Brul, S., & ter Kuile, B. H. (2014). Interaction between mutations and regulation of gene expression during development of de novo antibiotic resistance. *Antimicrobial agents and chemotherapy*, *58*(8), 4371-4379.

Handzlik, J., Matys, A., & Kieć-Kononowicz, K. (2013). Recent advances in multi-drug resistance (MDR) efflux pump inhibitors of Gram-positive bacteria S. aureus. *Antibiotics*, *2*(1), 28-45.

Hartemann-Heurtier, A., Robert, J., Jacqueminet, S., Ha Van, G., Golmard, J., Jarlier, V., & Grimaldi, A. (2004). Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact. *Diabetic Medicine*, *21*(7), 710-715.

Heilman, F. R., Herrell, W. E., Wellman, W. E., & Geraci, J. E. (1952). Some laboratory and clinical observations on a new antibiotic, erythromycin (ilotycin).

Hiramatsu, K., Ito, T., Tsubakishita, S., Sasaki, T., Takeuchi, F., Morimoto, Y., Katayama, Y., Matsuo, M., Kuwahara-Arai, K., & Hishinuma, T. (2013). Genomic basis for methicillin resistance in Staphylococcus aureus. *Infection & chemotherapy*, *45*(2), 117-136.

Holmkvist, J., Cervin, C., Lyssenko, V., Winckler, W., Anevski, D., Cilio, C., Almgren, P., Berglund, G., Nilsson, P., & Tuomi, T. (2006). Common variants in HNF-1 α and risk of type 2 diabetes. *Diabetologia*, *49*, 2882-2891.

Hong, S. H., Wang, X., O'Connor, H. F., Benedik, M. J., & Wood, T. K. (2012). Bacterial persistence increases as environmental fitness decreases. *Microbial biotechnology*, *5*(4), 509-522.

Howell-Jones, R. S., Price, P. E., Howard, A. J., & Thomas, D. W. (2006). Antibiotic prescribing for chronic skin wounds in primary care. *Wound repair and regeneration*, *14*(4), 387-393.

Hsu, C.-Y., Shu, J.-C., Lin, M.-H., Chong, K.-Y., Chen, C.-C., Wen, S.-M., Hsieh, Y.-T., & Liao, W.-T. (2015). High glucose concentration promotes vancomycin-enhanced biofilm formation of vancomycin-non-susceptible Staphylococcus aureus in diabetic mice. *PloS one*, *10*(8), e0134852.

Hu, Y., Liu, A., Ortega-Muro, F., Alameda-Martin, L., Mitchison, D., & Coates, A. (2015). High-dose rifampicin kills persisters, shortens treatment duration, and reduces relapse rate in vitro and in vivo. *Frontiers in microbiology*, *6*, 641.

Huether, M. J., Griego, R. D., Brodland, D. G., & Zitelli, J. A. (2002). Clindamycin for intraincisional antibiotic prophylaxis in dermatologic surgery. *Archives of dermatology*, *138*(9), 1145-1148.

Hussain, H. I., Aqib, A. I., Seleem, M. N., Shabbir, M. A., Hao, H., Iqbal, Z., Kulyar, M. F.-e.-A., Zaheer, T., & Li, K. (2021). Genetic basis of molecular mechanisms in β-lactam resistant gram-negative bacteria. *Microbial pathogenesis*, *158*, 105040.

Imayama, I., Plotnikoff, R. C., Courneya, K. S., & Johnson, J. A. (2011). Determinants of quality of life in adults with type 1 and type 2 diabetes. *Health and quality of life outcomes*, *9*, 1-9.

Jemal, A., Ward, E. M., Johnson, C. J., Cronin, K. A., Ma, J., Ryerson, A. B., Mariotto, A., Lake, A. J., Wilson, R., & Sherman, R. L. (2017). Annual report to the nation on the status of cancer, 1975–2014, featuring survival. *JNCI: Journal of the National Cancer Institute*, *109*(9), djx030.

Jensen, S. O., & Lyon, B. R. (2009). Genetics of antimicrobial resistance in Staphylococcus aureus. *Future microbiology*, *4*(5), 565-582.

Jernigan, J. A., Hatfield, K. M., Wolford, H., Nelson, R. E., Olubajo, B., Reddy, S. C., McCarthy, N., Paul, P., McDonald, L. C., & Kallen, A. (2020). Multidrug-resistant bacterial infections in US hospitalized patients, 2012–2017. *New England Journal of Medicine*, *382*(14), 1309-1319.

Jo, J.-H., Harkins, C. P., Schwardt, N. H., Portillo, J. A., Program, N. C. S., Zimmerman, M. D., Carter, C. L., Hossen, M. A., Peer, C. J., & Polley, E. C. (2021). Alterations of human skin microbiome and expansion of antimicrobial resistance after systemic antibiotics. *Science translational medicine*, *13*(625), eabd8077.

Jupiter, D. C., Thorud, J. C., Buckley, C. J., & Shibuya, N. (2016). The impact of foot ulceration and amputation on mortality in diabetic patients. I: From ulceration to death, a systematic review. *International Wound Journal*, *13*(5), 892-903.

Kaba, H. E., Kuhlmann, E., & Scheithauer, S. (2020). Thinking outside the box: Association of antimicrobial resistance with climate warming in Europe–A 30 country observational study. *International journal of hygiene and environmental health*, *223*(1), 151-158.

Kalan, L. R., Meisel, J. S., Loesche, M. A., Horwinski, J., Soaita, I., Chen, X., Uberoi, A., Gardner, S. E., & Grice, E. A. (2019). Strain-and species-level variation in the microbiome of diabetic wounds is associated with clinical outcomes and therapeutic efficacy. *Cell host & microbe*, *25*(5), 641-655. e645.

Kasten, M. J. (1999). Clindamycin, metronidazole, and chloramphenicol. Mayo Clinic Proceedings,

Kathirvel, M., Jayarajan, J., Sivakumar, A., & Govindan, V. (2018). Risk factors for the diabetic foot infection with multidrug-resistant microorganisms in South India. *International Surgery Journal*, *5*(2), 675-682.

Khan, K. I., Akmal, M., Waqas, A., & Mahmood, S. (2014). Role of prophylactic antibiotics in Milligan Morgan hemorrhoidectomy–a randomized control trial. *International Journal of Surgery*, *12*(8), 868-871.

Kurepina, N., Chudaev, M., Kreiswirth, B. N., Nikiforov, V., & Mustaev, A. (2022). Mutations compensating for the fitness cost of rifampicin resistance in Escherichia coli exert pleiotropic effect on RNA polymerase catalysis. *Nucleic acids research*, *50*(10), 5739-5756.

Kurokawa, I., Nishijima, S., & Asada, Y. (1988). The antibiotic susceptibility of Propionibacterium acnes: a 15-year bacteriological study and retrospective evaluation. *The Journal of Dermatology*, *15*(2), 149-154.

Kurow, O., Nuwayhid, R., Stock, P., Steinert, M., Langer, S., Krämer, S., & Metelmann, I. B. (2023). Organotypic 3D Co-Culture of Human Pleura as a Novel In Vitro Model of Staphylococcus aureus Infection and Biofilm Development. *Bioengineering*, *10*(5), 537.

Laub, R., Schneider, Y.-J., & Trouet, A. (1989). Antibiotic susceptibility of Salmonella spp. at different pH values. *Microbiology*, *135*(6), 1407-1416.

Le, K. Y., Villaruz, A. E., Zheng, Y., He, L., Fisher, E. L., Nguyen, T. H., Ho, T. V., Yeh, A. J., Joo, H.-S., & Cheung, G. Y. (2019). Role of phenol-soluble modulins in Staphylococcus epidermidis biofilm formation and infection of indwelling medical devices. *Journal of molecular biology*, *431*(16), 3015-3027.

Lee, J. Y., Monk, I. R., Gonçalves da Silva, A., Seemann, T., Chua, K. Y., Kearns, A., Hill, R., Woodford, N., Bartels, M. D., & Strommenger, B. (2018). Global spread of three multidrug-resistant lineages of Staphylococcus epidermidis. *Nature microbiology*, *3*(10), 1175-1185.

Lengheden, A., & Jansson, L. (1995). pH effects on experimental wound healing of human fibroblasts in vitro. *European journal of oral sciences*, *103*(3), 148-155.

Leveen, H. H., Falk, G., Borek, B., Diaz, C., Lynfield, Y., Wynkoop, B. J., Mabunda, G. A., Rubricius, J. L., & Christoudias, G. C. (1973). Chemical acidification of wounds. An adjuvant to healing and the unfavorable action of alkalinity and ammonia. *Annals of surgery*, *178*(6), 745.

Li, D., Wang, W., Wu, Y., Ma, X., Zhou, W., & Lai, Y. (2019). Lipopeptide 78 from Staphylococcus epidermidis activates β-catenin to inhibit skin inflammation. *The Journal of Immunology*, *202*(4), 1219-1228.

Li, M. M., Ray, P., Teets, C., Pruden, A., Xia, K., & Knowlton, K. F. (2020). Increasing temperature and pH can facilitate reductions of cephapirin and antibiotic resistance genes in dairy manure slurries. *Journal of dairy science*, *103*(3), 2877-2882.

Li, W., Liu, C., Ho, H. C., Shi, L., Zeng, Y., Yang, X., Huang, Q., Pei, Y., Huang, C., & Yang, L. (2023). Association between antibiotic resistance and increasing ambient temperature in China: an ecological study with nationwide panel data. *The Lancet Regional Health–Western Pacific*, *30*.

Liang, J.-H., & Han, X. (2013). Structure-activity relationships and mechanism of action of macrolides derived from erythromycin as antibacterial agents. *Current Topics in Medicinal Chemistry*, *13*(24), 3131-3164.

Lim, S.-K., Lee, S.-O., Choi, S.-H., Choi, J.-P., Kim, S.-H., Jeong, J.-Y., Choi, S.-H., Woo, J. H., & Kim, Y. S. (2011). The outcomes of using colistin for treating multidrug resistant Acinetobacter species bloodstream infections. *Journal of Korean medical science*, *26*(3), 325-331.

Lin, Q., Pilewski, J. M., & Di, Y. P. (2021). Acidic microenvironment determines antibiotic susceptibility and biofilm formation of Pseudomonas aeruginosa. *Frontiers in microbiology*, *12*, 747834.

Lipsky, B. A., Berendt, A. R., Cornia, P. B., Pile, J. C., Peters, E. J., Armstrong, D. G., Deery, H. G., Embil, J. M., Joseph, W. S., & Karchmer, A. W. (2012). 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clinical Infectious Diseases*, *54*(12), e132-e173.

Liu, G. Y., Essex, A., Buchanan, J. T., Datta, V., Hoffman, H. M., Bastian, J. F., Fierer, J., & Nizet, V. (2005). Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *The Journal of experimental medicine*, *202*(2), 209-215.

Liu, H., Taylor, T. H., Pettus, K., Johnson, S., Papp, J. R., & Trees, D. (2016). Comparing the disk-diffusion and agar dilution tests for Neisseria gonorrhoeae antimicrobial susceptibility testing. *Antimicrobial Resistance & Infection Control*, *5*, 1-6.

Liu, M. Y., Cokcetin, N. N., Lu, J., Turnbull, L., Carter, D. A., Whitchurch, C. B., & Harry, E. J. (2018). Rifampicin-manuka honey combinations are superior to other antibiotic-manuka honey combinations in eradicating Staphylococcus aureus biofilms. *Frontiers in microbiology*, *8*, 2653.

López, A. d. L. R., Lee, M.-R., Ortiz, B. J., Gastfriend, B. D., Whitehead, R., Lynn, D. M., & Palecek, S. P. (2019). Preventing S. aureus biofilm formation on titanium surfaces by

the release of antimicrobial β-peptides from polyelectrolyte multilayers. *Acta biomaterialia*, *93*, 50-62.

Lu, X., Samuelson, D. R., Xu, Y., Zhang, H., Wang, S., Rasco, B. A., Xu, J., & Konkel, M. E. (2013). Detecting and tracking nosocomial methicillin-resistant Staphylococcus aureus using a microfluidic SERS biosensor. *Analytical chemistry*, *85*(4), 2320-2327.

Lundberg, C. V., & Frimodt-Møller, N. (2013). Efficacy of topical and systemic antibiotic treatment of meticillin-resistant Staphylococcus aureus in a murine superficial skin wound infection model. *International journal of antimicrobial agents*, *42*(3), 272-275.

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S., Hindler, J., Kahlmeter, G., & Olsson-Liljequist, B. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268-281.

Martínez, J. L., & Rojo, F. (2011). Metabolic regulation of antibiotic resistance. *FEMS microbiology reviews*, *35*(5), 768-789.

McArdle, C. D., Lagan, K. M., & McDowell, D. A. (2018). Effects of pH on the antibiotic resistance of bacteria recovered from diabetic foot ulcer fluid: an in vitro study. *Journal of the American Podiatric Medical Association*, *108*(1), 6-11.

McCourt, J., O'Halloran, D. P., McCarthy, H., O'Gara, J. P., & Geoghegan, J. A. (2014). Fibronectin-binding proteins are required for biofilm formation by community-associated methicillin-resistant Staphylococcus aureus strain LAC. *FEMS microbiology letters*, *353*(2), 157-164.

Meloni, M., Izzo, V., Giurato, L., Lázaro-Martínez, J. L., & Uccioli, L. (2020). Prevalence, clinical aspects and outcomes in a large cohort of persons with diabetic foot disease: comparison between neuropathic and ischemic ulcers. *Journal of clinical medicine*, *9*(6), 1780.

Meneghetti, K. L., do Canto Canabarro, M., Otton, L. M., dos Santos Hain, T., Geimba, M. P., & Corção, G. (2018). Bacterial contamination of human skin allografts and antimicrobial resistance: a skin bank problem. *BMC microbiology*, *18*, 1-9.

Meneilly, G. S. (2000). Pathophysiology of diabetes in the elderly. *Diabetes in old age*, 17-23.

Mercier, R.-C., Stumpo, C., & Rybak, M. J. (2002). Effect of growth phase and pH on the in vitro activity of a new glycopeptide, oritavancin (LY333328), against Staphylococcus aureus and Enterococcus faecium. *Journal of Antimicrobial Chemotherapy*, *50*(1), 19-24.

Miladiyah, I., & Rachmawaty, F. J. (2017). Potency of xanthone derivatives as antibacterial agent against Methicillin-Resistant Staphylococcus Aureus (MRSA). *JKKI: Jurnal Kedokteran dan Kesehatan Indonesia*, 124-135.

Mirhoseini, M., Kianezhad, M. A., Rezaeipour, B., Ghasemi, M., Rezanejad Gatabi, Z., Nia, H. S., & Talebpour Amiri, F. (2021). The synergistic effect of topical insulin and clindamycin on acute dermal wound healing in rat model: a histological study. *Journal of Histotechnology*, *44*(2), 70-79.

Mirhoseini, S. H., Nikaeen, M., Shamsizadeh, Z., & Khanahmad, H. (2016). Hospital air: A potential route for transmission of infections caused by β-lactam–resistant bacteria. *American journal of infection control*, *44*(8), 898-904.

Moulik, P. K., Mtonga, R., & Gill, G. V. (2003). Amputation and mortality in new-onset diabetic foot ulcers stratified by etiology. *Diabetes care*, *26*(2), 491-494.

Muduli, I. C., PP, A., Panda, C., & Behera, N. C. (2015). Diabetic foot ulcer complications and its management—a medical college-based descriptive study in Odisha, an Eastern State of India. *Indian Journal of Surgery*, *77*, 270-274.

Muhlebach, M. S., Beckett, V., Popowitch, E., Miller, M. B., Baines, A., Mayer-Hamblett, N., Zemanick, E. T., Hoover, W. C., VanDalfsen, J. M., & Campbell, P. (2017). Microbiological efficacy of early MRSA treatment in cystic fibrosis in a randomised controlled trial. *Thorax*, *72*(4), 318-326.

Müller, P., Alber, D. G., Turnbull, L., Schlothauer, R. C., Carter, D. A., Whitchurch, C. B., & Harry, E. J. (2013). Synergism between Medihoney and rifampicin against methicillin-resistant Staphylococcus aureus (MRSA). *PloS one*, *8*(2), e57679.

Murugan, S., Mani, K., & Uma Devi, P. (2008). Prevalence of methicillin resistant Staphylococcus aureus among diabetes patients with foot ulcers and their antimicrobial susceptibility pattern. *Journal of Clinical and Diagnostic Research*, *2*, 979-984.

Nakagami, G., Sanada, H., Iizaka, S., Kadono, T., Higashino, T., Koyanagi, H., & Haga, N. (2010). Predicting delayed pressure ulcer healing using thermography: a prospective cohort study. *Journal of wound care*, *19*(11), 465-472.

Nakagami, G., Sari, Y., Nagase, T., Iizaka, S., Ohta, Y., & Sanada, H. (2010). Evaluation of the usefulness of skin blood flow measurements by laser speckle flowgraphy in pressure-induced ischemic wounds in rats. *Annals of plastic surgery*, *64*(3), 351-354.

Nakaminami, H., Tajima, M., Koishikawa, K., Shiratori, Y., Shioura, M., Sasatsu, M., & Noguchi, N. (2019). Development of effective antimicrobial cocktails to prevent bacterial contamination of allograft tissues under low temperature conditions. *Interactive CardioVascular and Thoracic Surgery*, *28*(1), 128-136.

Obolski, U., Dellus-Gur, E., Stein, G. Y., & Hadany, L. (2016). Antibiotic cross-resistance in the lab and resistance co-occurrence in the clinic: discrepancies and implications in E. coli. *Infection, Genetics and Evolution*, *40*, 155-161.

Öien, R. F., & Forssell, H. W. (2013). Ulcer healing time and antibiotic treatment before and after the introduction of the Registry of Ulcer Treatment: an improvement project in a national quality registry in Sweden. *BMJ open*, *3*(8), e003091.

Oliveira, A. C. d., Rocha, D. d. M., Bezerra, S. M. G., Andrade, E. M. L. R., Santos, A. M. R. d., & Nogueira, L. T. (2019). Quality of life of people with chronic wounds. *Acta Paulista de Enfermagem*, *32*, 194-201.

Ono, S., Imai, R., Ida, Y., Shibata, D., Komiya, T., & Matsumura, H. (2015). Increased wound pH as an indicator of local wound infection in second degree burns. *Burns*, *41*(4), 820-824.

Otto, M. (2009). Staphylococcus epidermidis—the'accidental'pathogen. *Nature reviews microbiology*, *7*(8), 555-567.

Oz, T., Guvenek, A., Yildiz, S., Karaboga, E., Tamer, Y. T., Mumcuyan, N., Ozan, V. B., Senturk, G. H., Cokol, M., & Yeh, P. (2014). Strength of selection pressure is an important parameter contributing to the complexity of antibiotic resistance evolution. *Molecular biology and evolution*, *31*(9), 2387-2401.

Packer, C. F., Ali, S. A., & Manna, B. (2024). Diabetic Ulcer. In *StatPearls*. StatPearls Publishing

Pál, C., Papp, B., & Lázár, V. (2015). Collateral sensitivity of antibiotic-resistant microbes. *Trends in microbiology*, *23*(7), 401-407.

Pandey, N., & Cascella, M. (2019). Beta lactam antibiotics.

Pankey, G. A., & Sabath, L. (2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clinical Infectious Diseases*, *38*(6), 864-870.

Patel, J. D., Ebert, M., Ward, R., & Anderson, J. M. (2007). S. epidermidis biofilm formation: effects of biomaterial surface chemistry and serum proteins. *Journal of Biomedical Materials Research Part A*, *80*(3), 742-751.

Paul, M., Lador, A., Grozinsky-Glasberg, S., & Leibovici, L. (2014). Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. *Cochrane Database of Systematic Reviews*(1).

Peng, B., Su, Y.-b., Li, H., Han, Y., Guo, C., Tian, Y.-m., & Peng, X.-x. (2015). Exogenous alanine and/or glucose plus kanamycin kills antibiotic-resistant bacteria. *Cell metabolism*, *21*(2), 249-262.

Percival, S. L., McCarty, S., Hunt, J. A., & Woods, E. J. (2014). The effects of pH on wound healing, biofilms, and antimicrobial efficacy. *Wound repair and regeneration*, *22*(2), 174-186.

Percival, S. L., Thomas, J. G., Slone, W., Linton, S., Corum, L., & Okel, T. (2011). The efficacy of silver dressings and antibiotics on MRSA and MSSA isolated from burn patients. *Wound repair and regeneration*, *19*(6), 767-774.

Perebikovsky, A., Liu, Y., Hwu, A., Kido, H., Shamloo, E., Song, D., Monti, G., Shoval, O., Gussin, D., & Madou, M. (2021). Rapid sample preparation for detection of antibiotic resistance on a microfluidic disc platform. *Lab on a Chip*, *21*(3), 534-545.

Pérez-Prieto, D., Portillo, M. E., González-Lucena, G., & Ginés-Cespedosa, A. (2019). Foot and ankle infections: debridement, early fixation and rifampicin provide earlier recovery of function and quality of life. *Foot and Ankle Surgery*, *25*(1), 13-18.

Pipelzadeh, M. H., & Naylor, I. L. (1998). The in vitro enhancement of rat myofibroblast contractility by alterations to the pH of the physiological solution. *European journal of pharmacology*, *357*(2-3), 257-259.

Pleeging, C. C., Coenye, T., Mossialos, D., De Rooster, H., Chrysostomou, D., Wagener, F. A., & Cremers, N. A. (2020). Synergistic antimicrobial activity of supplemented medical-grade honey against Pseudomonas aeruginosa biofilm formation and eradication. *Antibiotics*, *9*(12), 866.

Pouget, C., Gustave, C.-A., Ngba-Essebe, C., Laurent, F., Lemichez, E., Tristan, A., Sotto, A., Dunyach-Rémy, C., & Lavigne, J.-P. (2021). Adaptation of Staphylococcus aureus in a medium mimicking a diabetic foot environment. *Toxins*, *13*(3), 230.

Prax, M., Mechler, L., Weidenmaier, C., & Bertram, R. (2016). Glucose augments killing efficiency of daptomycin challenged Staphylococcus aureus persisters. *PloS one*, *11*(3), e0150907.

Price, N. (2020). Routine fluorescence imaging to detect wound bacteria reduces antibiotic use and antimicrobial dressing expenditure while improving healing rates: Retrospective analysis of 229 foot ulcers. *Diagnostics*, *10*(11), 927.

Prompers, L., Schaper, N., Apelqvist, J., Edmonds, M., Jude, E., Mauricio, D., Uccioli, L., Urbancic, V., Bakker, K., & Holstein, P. (2008). Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. *Diabetologia*, *51*, 747-755.

Qi, Q., Toll-Riera, M., Heilbron, K., Preston, G. M., & MacLean, R. C. (2016). The genomic basis of adaptation to the fitness cost of rifampicin resistance in Pseudomonas aeruginosa. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1822), 20152452.

Quave, C. L., Estevez-Carmona, M., Compadre, C. M., Hobby, G., Hendrickson, H., Beenken, K. E., & Smeltzer, M. S. (2012). Ellagic acid derivatives from Rubus ulmifolius inhibit Staphylococcus aureus biofilm formation and improve response to antibiotics. *PloS one*, *7*(1), e28737.

Rasigade, J.-P., Dumitrescu, O., & Lina, G. (2014). New epidemiology of Staphylococcus aureus infections. *Clinical Microbiology and Infection*, *20*(7), 587-588.

Reinbold, J., Hierlemann, T., Urich, L., Uhde, A.-K., Müller, I., Weindl, T., Vogel, U., Schlensak, C., Wendel, H. P., & Krajewski, S. (2017). Biodegradable rifampicin-releasing coating of surgical meshes for the prevention of bacterial infections. *Drug Design, Development and Therapy*, 2753-2762.

Rodríguez-Verdugo, A., Gaut, B. S., & Tenaillon, O. (2013). Evolution of Escherichia coli rifampicin resistance in an antibiotic-free environment during thermal stress. *BMC evolutionary biology*, *13*, 1-11.

Rogers, G. B., Bruce, K. D., Martin, M. L., Burr, L. D., & Serisier, D. J. (2014). The effect of long-term macrolide treatment on respiratory microbiota composition in non-cystic fibrosis bronchiectasis: an analysis from the randomised, double-blind, placebo-controlled BLESS trial. *The lancet Respiratory medicine*, *2*(12), 988-996.

Rohde, H., Burdelski, C., Bartscht, K., Hussain, M., Buck, F., Horstkotte, M. A., Knobloch, J. K. M., Heilmann, C., Herrmann, M., & Mack, D. (2005). Induction of Staphylococcus epidermidis biofilm formation via proteolytic processing of the accumulation-associated protein by staphylococcal and host proteases. *Molecular microbiology*, *55*(6), 1883-1895.

Ross, A. A., Müller, K. M., Weese, J. S., & Neufeld, J. D. (2018). Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phylosymbiosis within the class Mammalia. *Proceedings of the National Academy of Sciences*, *115*(25), E5786-E5795.

Roy, S., Santra, S., Das, A., Dixith, S., Sinha, M., Ghatak, S., Ghosh, N., Banerjee, P., Khanna, S., & Mathew-Steiner, S. (2020). Staphylococcus aureus biofilm infection compromises wound healing by causing deficiencies in granulation tissue collagen. *Annals of surgery*, *271*(6), 1174-1185.

Sahal, G., & Bilkay, I. S. (2014). Multi drug resistance in strong biofilm forming clinical isolates of Staphylococcus epidermidis. *Brazilian Journal of Microbiology*, *45*, 539-544.

Samir, S., El-Far, A., Okasha, H., Mahdy, R., Samir, F., & Nasr, S. (2022). Isolation and characterization of lytic bacteriophages from sewage at an egyptian tertiary care hospital against methicillin-resistant Staphylococcus aureus clinical isolates. *Saudi Journal of Biological Sciences*, *29*(5), 3097-3106.

Santimaleeworagun, W., Wongpoowarak, P., Chayakul, P., Pattharachayakul, S., Tansakul, P., & Garey, K. W. (2011). In vitro activity of colistin or sulbactam in combination with fosfomycin or imipenem against clinical isolates of carbapenem-resistant Acinetobacter baumannii producing OXA-23 carbapenemases. *Southeast Asian Journal of Tropical Medicineand Public Health*, *42*(4), 890.

Santos-Lopez, A., Marshall, C. W., Scribner, M. R., Snyder, D. J., & Cooper, V. S. (2019). Evolutionary pathways to antibiotic resistance are dependent upon environmental structure and bacterial lifestyle. *Elife*, *8*, e47612.

Scheinfeld, N. (2016). Why rifampin (rifampicin) is a key component in the antibiotic treatment of hidradenitis suppurativa: a review of rifampin's effects on bacteria, bacterial biofilms, and the human immune system. *Dermatology Online Journal*, *22*(6).

Scribner, M., Santos-Lopez, A., Marshall, C., Deitrick, C., & Cooper, V. (2020). Parallel evolution of tobramycin resistance across species and environments. mBio 11: e00932-20. In.

Serra, R., Grande, R., Butrico, L., Rossi, A., Settimio, U. F., Caroleo, B., Amato, B., Gallelli, L., & De Franciscis, S. (2015). Chronic wound infections: the role of Pseudomonas aeruginosa and Staphylococcus aureus. *Expert review of anti-infective therapy*, *13*(5), 605-613.

She, P., Wang, Y., Liu, Y., Tan, F., Chen, L., Luo, Z., & Wu, Y. (2019). Effects of exogenous glucose on Pseudomonas aeruginosa biofilm formation and antibiotic resistance. *Microbiologyopen*, *8*(12), e933.

Sim, P., Strudwick, X. L., Song, Y., Cowin, A. J., & Garg, S. (2022). Influence of Acidic pH on Wound Healing In Vivo: A Novel Perspective for Wound Treatment. *Int J Mol Sci*, *23*(21). https://doi.org/10.3390/ijms232113655

Sim, P., Strudwick, X. L., Song, Y., Cowin, A. J., & Garg, S. (2022). Influence of acidic pH on wound healing in vivo: a novel perspective for wound treatment. *International Journal of Molecular Sciences*, *23*(21), 13655.

Singh, A. K., & Bhunia, A. K. (2019). Animal-use antibiotics induce cross-resistance in bacterial pathogens to human therapeutic antibiotics. *Current microbiology*, *76*, 1112-1117.

Skinner, K., Sandoe, J. A., Rajendran, R., Ramage, G., & Lang, S. (2017). Efficacy of rifampicin combination therapy for the treatment of enterococcal infections assessed in vivo using a Galleria mellonella infection model. *International journal of antimicrobial agents*, *49*(4), 507-511.

Smith, R. P., Baltch, A. L., Franke, M. A., Michelsen, P. B., & Bopp, L. H. (2000). Levofloxacin penetrates human monocytes and enhances intracellular killing of Staphylococcus aureus and Pseudomonas aeruginosa. *Journal of Antimicrobial Chemotherapy*, *45*(4), 483-488.

Sonbol, F. I., El-Banna, T., Abd El-Aziz, A. A., & El-Ekhnawy, E. (2019). Impact of triclosan adaptation on membrane properties, efflux and antimicrobial resistance of Escherichia coli clinical isolates. *Journal of applied microbiology*, *126*(3), 730-739.

Spagnolo, F., Rinaldi, C., Sajorda, D. R., & Dykhuizen, D. E. (2016). Evolution of resistance to continuously increasing streptomycin concentrations in populations of Escherichia coli. *Antimicrobial agents and chemotherapy*, *60*(3), 1336-1342.

Stavrakis, A. I., Niska, J. A., Shahbazian, J. H., Loftin, A. H., Ramos, R. I., Billi, F., Francis, K. P., Otto, M., Bernthal, N. M., & Uslan, D. Z. (2014). Combination prophylactic therapy with rifampin increases efficacy against an experimental Staphylococcus epidermidis subcutaneous implant-related infection. *Antimicrobial agents and chemotherapy*, *58*(4), 2377-2386.

Subramanian, P., Shanmugam, N., Sivaraman, U., Kumar, S., & Selvaraj, S. (2012). Antiobiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. *The Australasian medical journal*, *5*(7), 344.

Sun, D., Huang, A., Recchia, F. A., Cui, Y., Messina, E. J., Koller, A., & Kaley, G. (2001). Nitric oxide-mediated arteriolar dilation after endothelial deformation. *American Journal of Physiology-Heart and Circulatory Physiology*, *280*(2), H714-H721.

Talebi Bezmin Abadi, A., Rizvanov, A. A., Haertlé, T., & Blatt, N. L. (2019). World Health Organization report: current crisis of antibiotic resistance. *BioNanoScience*, *9*(4), 778-788.

Tang, B., Gong, T., Cui, Y., Wang, L., He, C., Lu, M., Chen, J., Jing, M., Zhang, A., & Li, Y. (2020). Characteristics of oral methicillin-resistant Staphylococcus epidermidis isolated from dental plaque. *International Journal of Oral Science*, *12*(1), 15.

Tavares, R. S. N., Tao, T. P., Maschmeyer, I., Maria-Engler, S. S., Schäfer-Korting, M., Winter, A., Zoschke, C., Lauster, R., Marx, U., & Gaspar, L. (2020). Toxicity of topically applied drugs beyond skin irritation: Static skin model vs. Two organs-on-a-chip. *International Journal of Pharmaceutics*, *589*, 119788.

Teng, Q.-X., Luo, X., Lei, Z.-N., Wang, J.-Q., Wurpel, J., Qin, Z., & Yang, D.-H. (2020). The multidrug resistance-reversing activity of a novel antimicrobial peptide. *Cancers*, *12*(7), 1963.

Tesauro, M., & Mazzotta, F. A. (2020). Pathophysiology of diabetes. In *Transplantation, bioengineering, and regeneration of the endocrine pancreas* (pp. 37-47). Elsevier.

Toprak, E., Veres, A., Michel, J.-B., Chait, R., Hartl, D. L., & Kishony, R. (2012). Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. *Nature genetics*, *44*(1), 101-105.

Truong-Bolduc, Q. C., Bolduc, G. R., Okumura, R., Celino, B., Bevis, J., Liao, C.-H., & Hooper, D. C. (2011). Implication of the NorB efflux pump in the adaptation of Staphylococcus aureus to growth at acid pH and in resistance to moxifloxacin. *Antimicrobial agents and chemotherapy*, *55*(7), 3214-3219.

Van Rie, A., Whitfield, M. G., De Vos, E., Scott, L., Da Silva, P., Hayes, C., Heupink, T. H., Sirgel, F. A., Stevens, W., & Warren, R. M. (2020). Discordances between molecular assays for rifampicin resistance in Mycobacterium tuberculosis: frequency, mechanisms and clinical impact. *Journal of Antimicrobial Chemotherapy*, *75*(5), 1123-1129.

Vogwill, T., Comfort, A., Furió, V., & MacLean, R. (2016). Persistence and resistance as complementary bacterial adaptations to antibiotics. *Journal of evolutionary biology*, *29*(6), 1223-1233.

Vogwill, T., Kojadinovic, M., Furió, V., & MacLean, R. C. (2014). Testing the role of genetic background in parallel evolution using the comparative experimental evolution of antibiotic resistance. *Molecular biology and evolution*, *31*(12), 3314-3323.

Wakamoto, Y., Dhar, N., Chait, R., Schneider, K., Signorino-Gelo, F., Leibler, S., & McKinney, J. D. (2013). Dynamic persistence of antibiotic-stressed mycobacteria. *Science*, *339*(6115), 91-95.

Walsh, C. T., & Wencewicz, T. A. (2014). Prospects for new antibiotics: a molecule-centered perspective. *The Journal of antibiotics*, *67*(1), 7-22.

Wanke, I., Steffen, H., Christ, C., Krismer, B., Götz, F., Peschel, A., Schaller, M., & Schittek, B. (2011). Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. *Journal of Investigative Dermatology*, *131*(2), 382-390.

Wareham-Mathiassen, S., Pinto Glenting, V., Bay, L., Allesen-Holm, M., Bengtsson, H., & Bjarnsholt, T. (2023). Characterization of pig skin microbiome and appraisal as an in vivo subcutaneous injection model. *Laboratory animals*, *57*(3), 304-318.

Wei, W., Liu, Q., Tan, Y., Liu, L., Li, X., & Cai, L. (2009). Oxidative stress, diabetes, and diabetic complications. *Hemoglobin*, *33*(5), 370-377.

Weinrick, B., Dunman, P. M., McAleese, F., Murphy, E., Projan, S. J., Fang, Y., & Novick, R. P. (2004). Effect of mild acid on gene expression in Staphylococcus aureus. *Journal of bacteriology*, *186*(24), 8407-8423.

Willer, C. J., Bonnycastle, L. L., Conneely, K. N., Duren, W. L., Jackson, A. U., Scott, L. J., Narisu, N., Chines, P. S., Skol, A., & Stringham, H. M. (2007). Screening of 134 single nucleotide polymorphisms (SNPs) previously associated with type 2 diabetes replicates association with 12 SNPs in nine genes. *Diabetes*, *56*(1), 256-264.

Wukich, D. K., Ahn, J., Raspovic, K. M., Gottschalk, F. A., La Fontaine, J., & Lavery, L. A. (2017). Comparison of transtibial amputations in diabetic patients with and without end-stage renal disease. *Foot & Ankle International*, *38*(4), 388-396.

Xiang, J., Wang, S.-w., Tao, Y., Ye, J.-z., Liang, Y., Peng, X.-x., Yang, L.-f., & Li, H. (2023). A glucose-mediated antibiotic resistance metabolic flux from glycolysis, the pyruvate

cycle, and glutamate metabolism to purine metabolism. *Frontiers in microbiology*, *14*, 1267729.

Xie, X., Bao, Y., Ni, L., Liu, D., Niu, S., Lin, H., Li, H., Duan, C., Yan, L., & Huang, S. (2017). Bacterial profile and antibiotic resistance in patients with diabetic foot ulcer in Guangzhou, Southern China: focus on the differences among different Wagner's grades, IDSA/IWGDF grades, and ulcer types. *International journal of endocrinology*, *2017*(1), 8694903.

Xue, H., Wu, Z., Qiao, D., Tong, C., & Zhao, X. (2017). Global acquisition of genetic material from different bacteria into the staphylococcal cassette chromosome elements of a Staphylococcus epidermidis isolate. *International journal of antimicrobial agents*, *50*(4), 581-587.

Yang, S., Hay, I. D., Cameron, D. R., Speir, M., Cui, B., Su, F., Peleg, A. Y., Lithgow, T., Deighton, M. A., & Qu, Y. (2015). Antibiotic regimen based on population analysis of residing persister cells eradicates Staphylococcus epidermidis biofilms. *Scientific reports*, *5*(1), 18578.

Zhang, M., Jiang, Z., Li, D., Jiang, D., Wu, Y., Ren, H., Peng, H., & Lai, Y. (2015). Oral antibiotic treatment induces skin microbiota dysbiosis and influences wound healing. *Microbial ecology*, *69*, 415-421.

Zhang, S., Yang, M. J., Peng, B., Peng, X. x., & Li, H. (2020). Reduced ROS-mediated antibiotic resistance and its reverting by glucose in Vibrio alginolyticus. *Environmental Microbiology*, *22*(10), 4367-4380.

Zhao, W., Zheng, S., Ye, C., Li, J., & Yu, X. (2024). Nonlinear Impacts of Temperature on Antibiotic Resistance in Escherichia coli. *Environmental Science and Ecotechnology*, 100475.

Zheng, Y., Hunt, R. L., Villaruz, A. E., Fisher, E. L., Liu, R., Liu, Q., Cheung, G. Y., Li, M., & Otto, M. (2022). Commensal Staphylococcus epidermidis contributes to skin barrier homeostasis by generating protective ceramides. *Cell host & microbe*, *30*(3), 301-313. e309.

Zhou, W., Spoto, M., Hardy, R., Guan, C., Fleming, E., Larson, P. J., Brown, J. S., & Oh, J. (2020). Host-specific evolutionary and transmission dynamics shape the functional diversification of Staphylococcus epidermidis in human skin. *Cell*, *180*(3), 454-470. e418.

Zhu, X., Wei, L., Rong, X., Zhang, Y., Zhang, Q., Wen, X., He, W., Zhang, K., Chen, F., & Wei, L. (2021). Conjunctival microbiota in patients with type 2 diabetes mellitus and influences of perioperative use of topical levofloxacin in ocular surgery. *Frontiers in Medicine*, *8*, 605639.

Zimmerli, W., Widmer, A. F., Blatter, M., Frei, R., & Ochsner, P. E. (1998). Role of rifampin for treatment of orthopedic implant–related staphylococcal infections: a randomized controlled trial. *Jama*, *279*(19), 1537-1541.